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NTERNATIONAL WEEKLY JOURNAL OF SCIENCE

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324

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CHROMATOGRAPHY/HPLC

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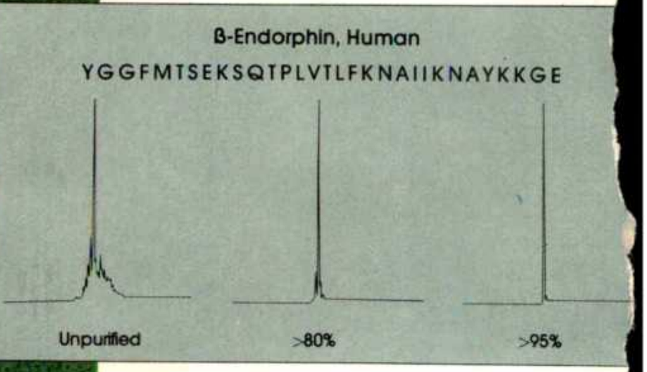

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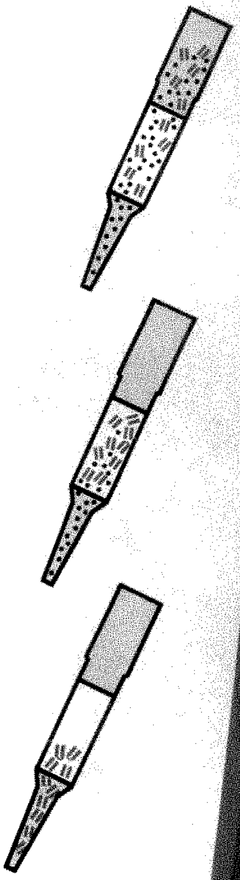

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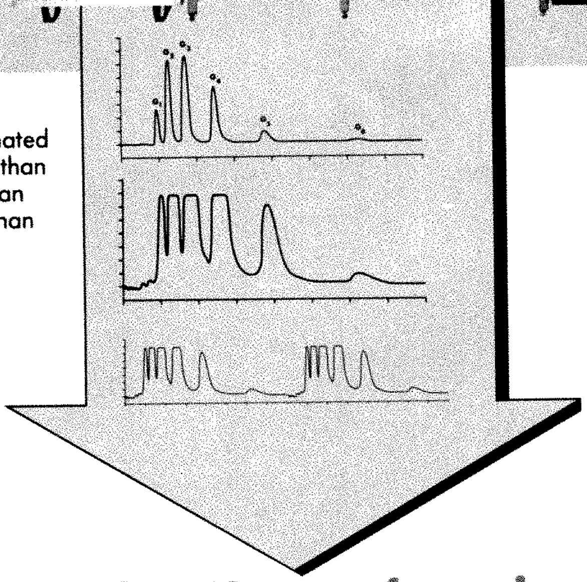

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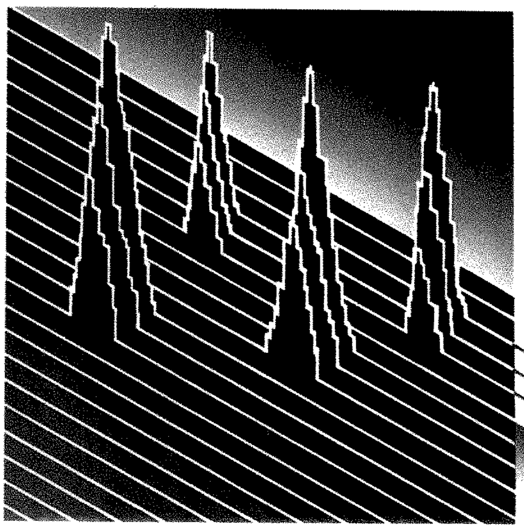

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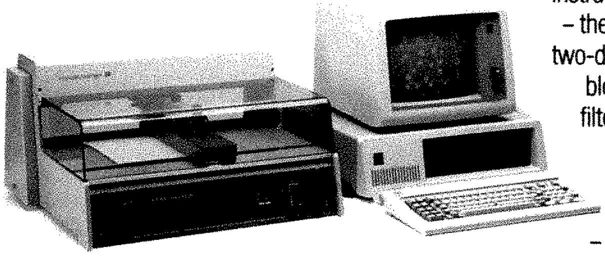

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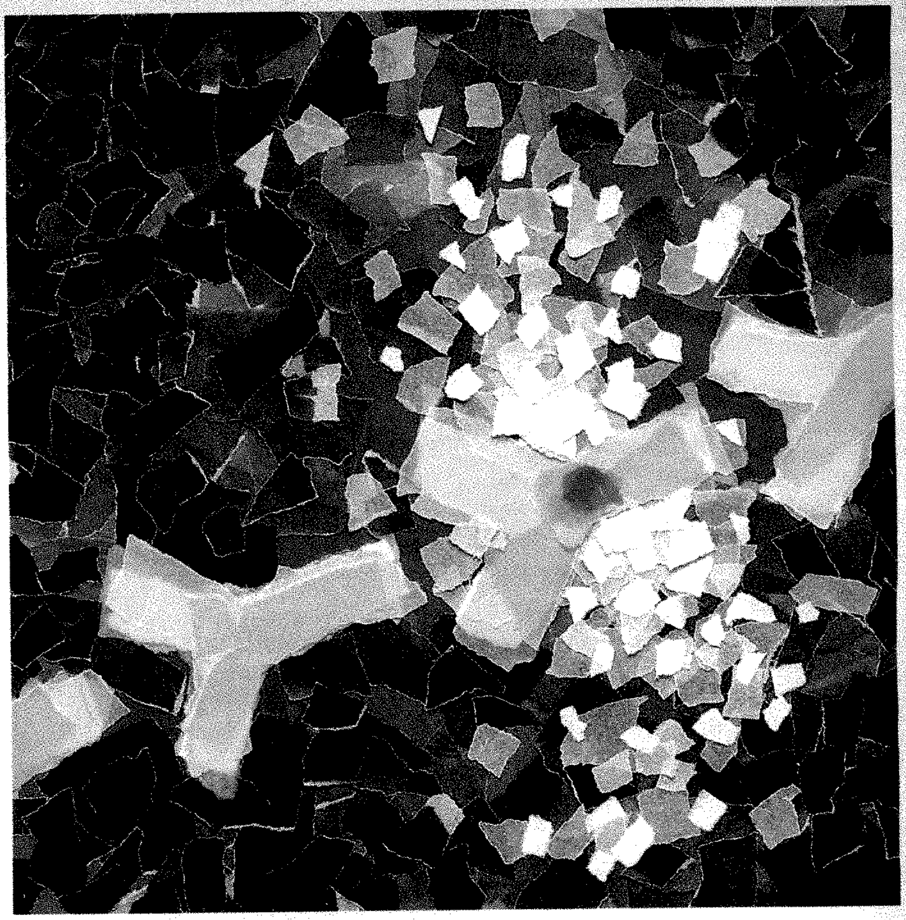

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5 July 1990

Vol. 346 Issue no. 6279

◀ The cotton-top tamarin is the most endangered primate in Colombia today. Endemic to northwest Colombia, an area of severe tropical rain forest destruction, the current population is between 300 and 1,000, though there are over 1,500 cotton-top tamarins in captivity. Study of the tamarin has thrown light on evolution of the immune systems of primates. Pages 60 and 17.

THIS WEEK . . . THIS WEEK . . . THIS WEEK . . .

On the rise

The conventional view that the high altitudes of most mountain ranges are the result of rapid late Cenozoic uplift, which in turn influenced the climate, is challenged in this week's Review Article. Molnar and England argue that global climate change may have preceded and helped cause the uplift. Page 29.

Genes in thrale

Agamous is a homeotic gene of the plant *Arabidopsis thaliana* (thrale cress): mutations of *agamous* cause transformation of stamens into petals, and replacement of carpels by a new flower. This gene has now been cloned and the sequence of its product reveals strong similarity to a class of DNA-binding regulatory proteins found in eukaryotes from yeast to man, and also the flower homeotic gene *deficiens* of *Antirrhinum*, mutations of which cause a different set of transformations. Pages 35 and 19.

Heated atmosphere

Lines of the bacterium *E. coli* maintained at an elevated temperature show rapid increases in fitness suggesting that at least some biological systems can adapt quickly to environmental change. Pages 79 and 15.

Where there's smoke

A 46-day study of a submarine thermal vent — a black smoker — reveals a remarkable temperature stability at 353±2 °C in the main flow of the hot spring, but a peak of 405 °C locally. These findings bear on a hotly debated topic, the temperature of fluids at depth beneath the sea floor. Page 51.

Yes to NO

Nitric oxide is not only the endothelium-derived factor that relaxes arterial smooth muscle but also the factor that causes hyperpolarization of the muscle, despite claims that a distinct substance is responsible. Page 69.

Prints of darkness

Cosmic ray sources, including the powerful γ-ray source Geminga, could be lumps of annihilating dark matter, according to the model proposed on page 39. The hypothesis that the free electrons that cause dispersion of pulsar signals result mainly from the decay of dark matter in our Galaxy is put to the test on page 40.

Pulsar triptych

The discovery of two new radio pulsars, the second and third in the globular cluster M15, suggests that there may be a large population of these objects, which may therefore be powerful indicators of the dynamics and evolution of globular clusters. Pages 42 and 12.

Body building

The *Krüppel* gene plays an important part in the development of the segmented body pattern of *Drosophila*, and the product of the gene has now been found to be able to 'actively' repress transcription. Page 76.

Anticlotting mechanism

Transgenic mice expressing the human gene for plasminogen inhibitor-1 develop venous occlusions, suggesting that the fibrinolytic activity of plasmin is continuously required to prevent fibrin deposition in veins. Page 74.

Hier et maintenant

Hulton

General de Gaulle, in his June 1940 BBC broadcast to the Free French. "Men of science" were also solicited to join the cause. See Then and Now.

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Late Cenozoic uplift of mountain ranges and global climate change: chicken or egg?
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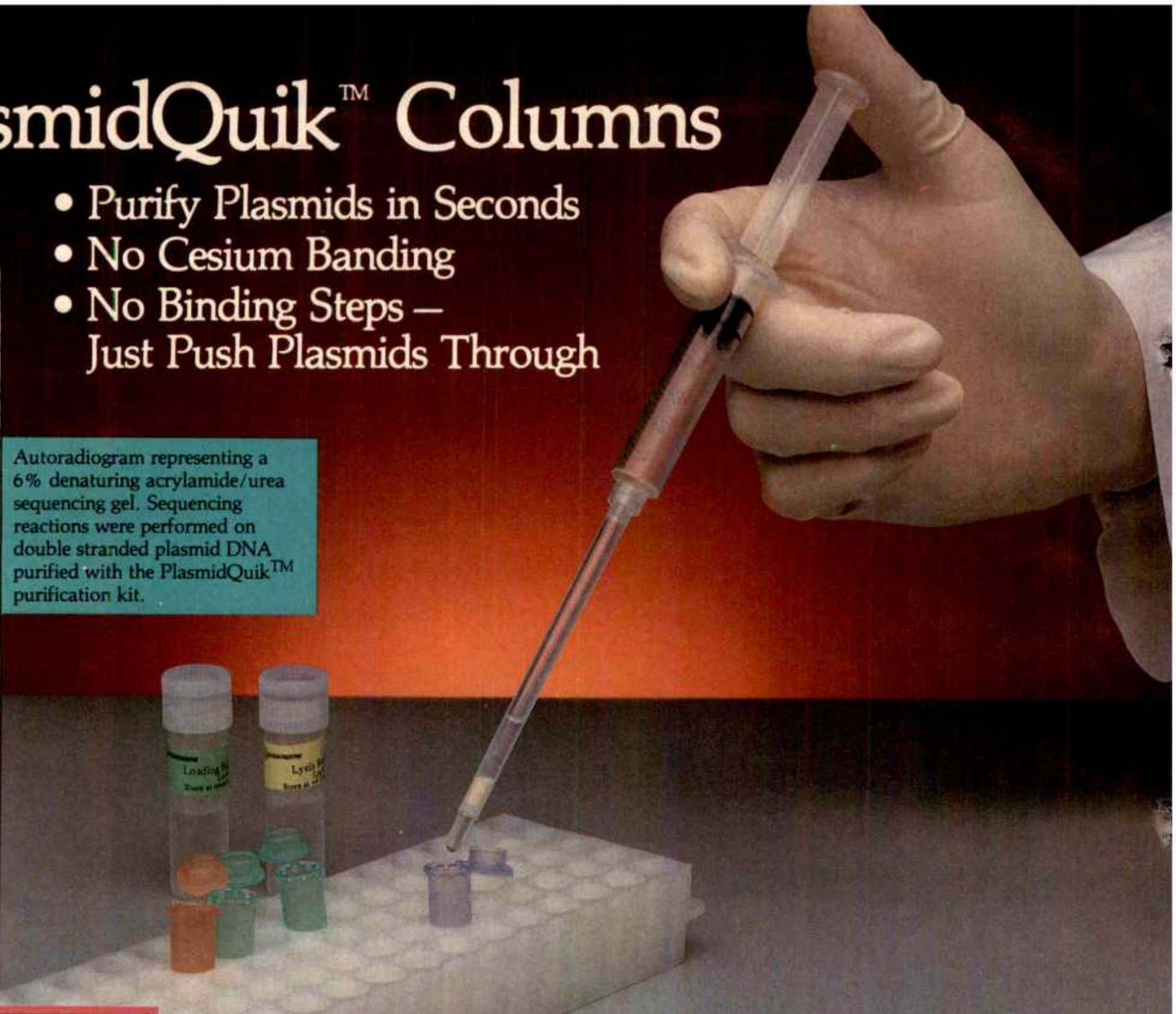
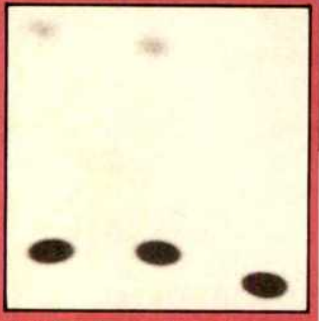


Autoradiogram representing a 6% denaturing acrylamide/urea sequencing gel. Sequencing reactions were performed on double stranded plasmid DNA purified with the PlasmidQuik™ purification kit.



Autoradiogram representing a CAT Assay/TLC Plate. COS-7 cells were transfected with 400 ng DNA purified with the PlasmidQuik™ purification kit (lane 1); 400 ng cesium banded DNA (lane 2); no DNA (lane 3). Cell transfections and the CAT assays were performed according to Stratagene protocols.

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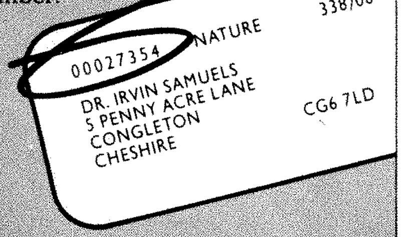

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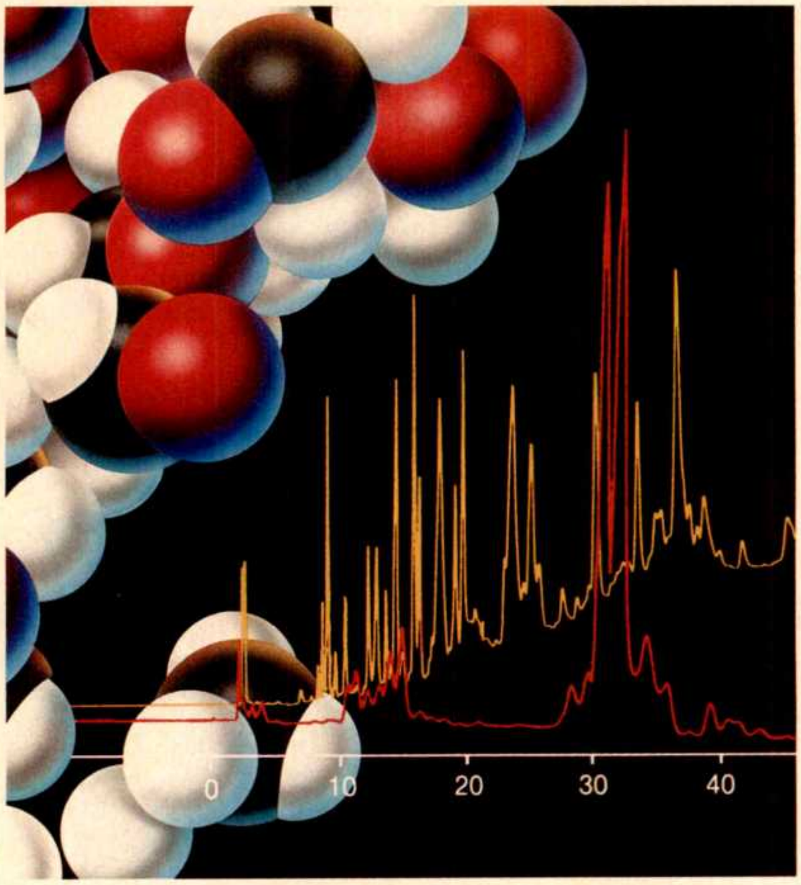

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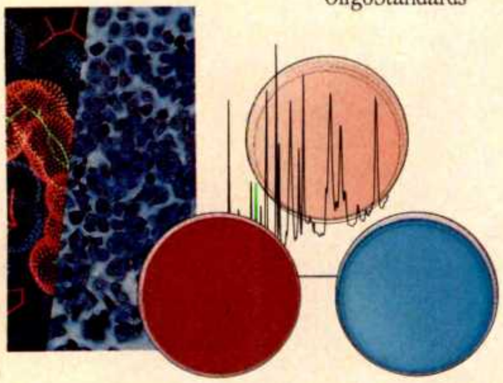

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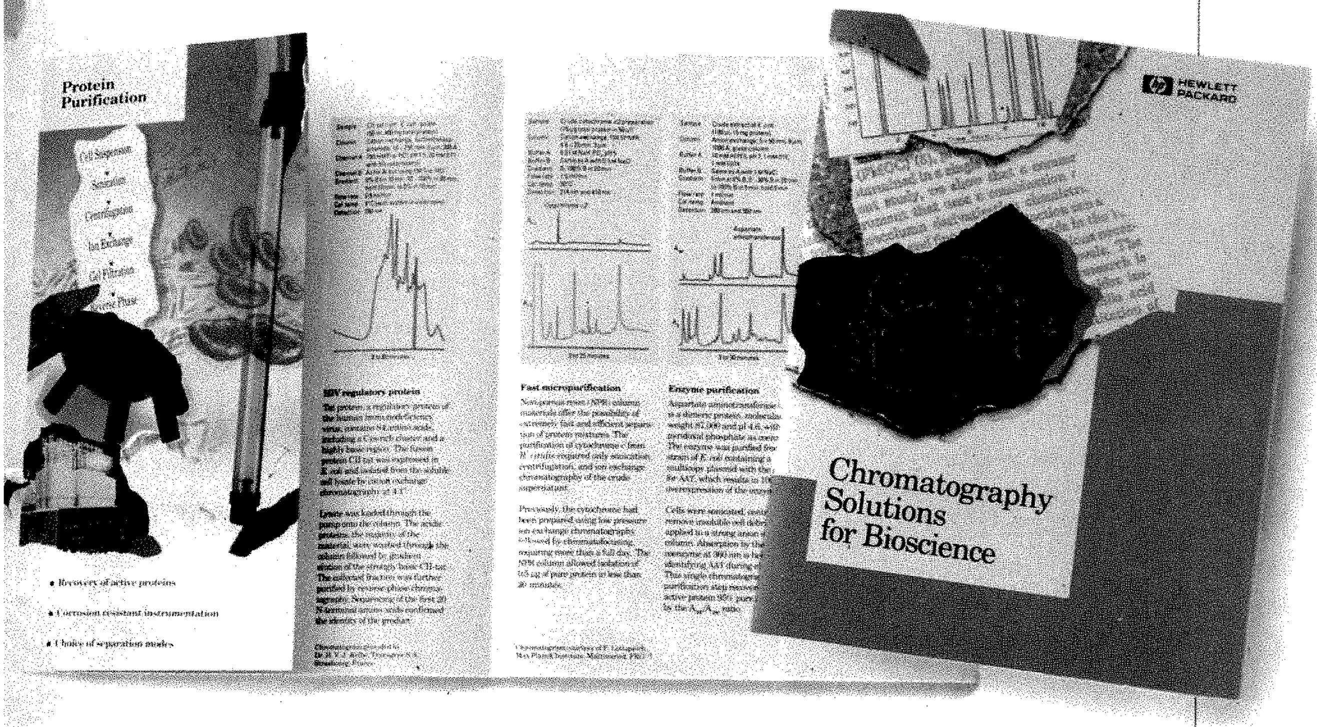

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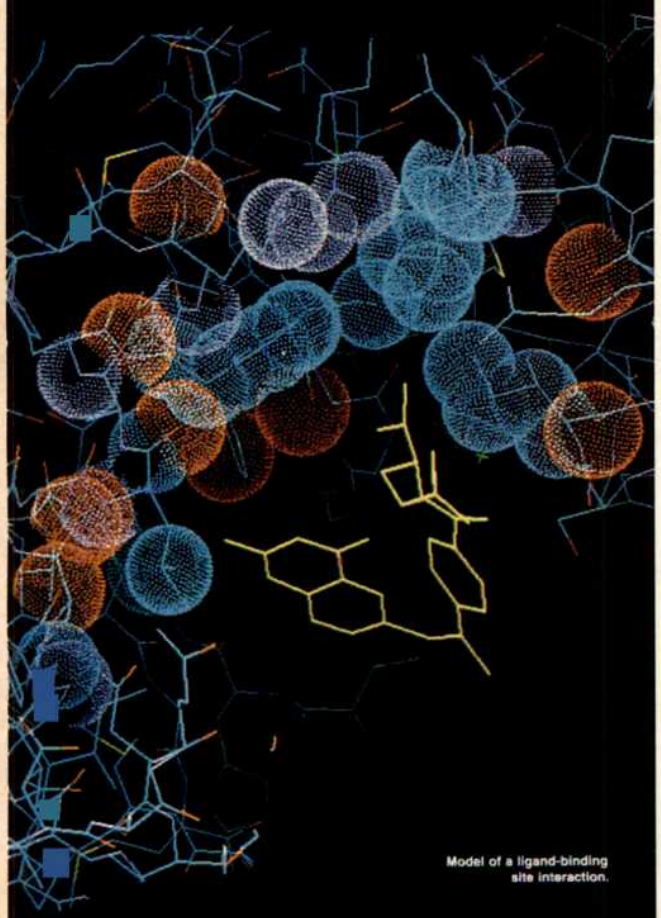

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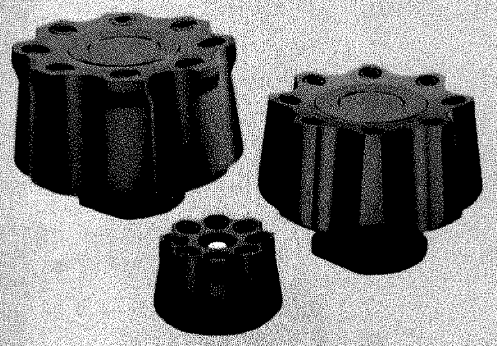

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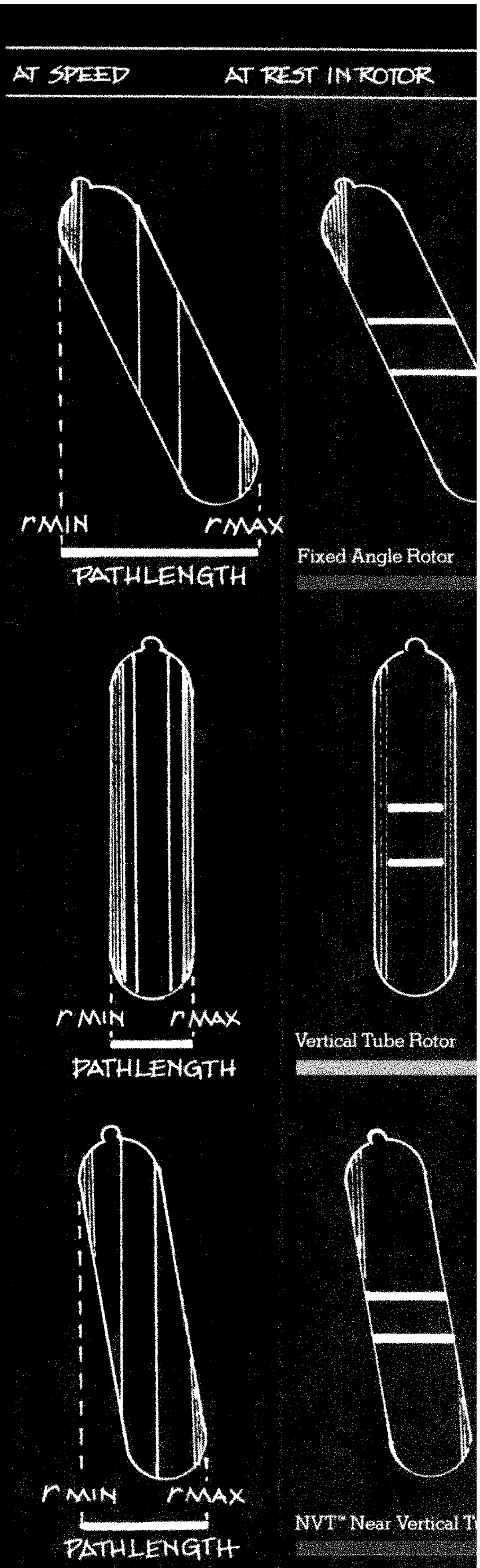

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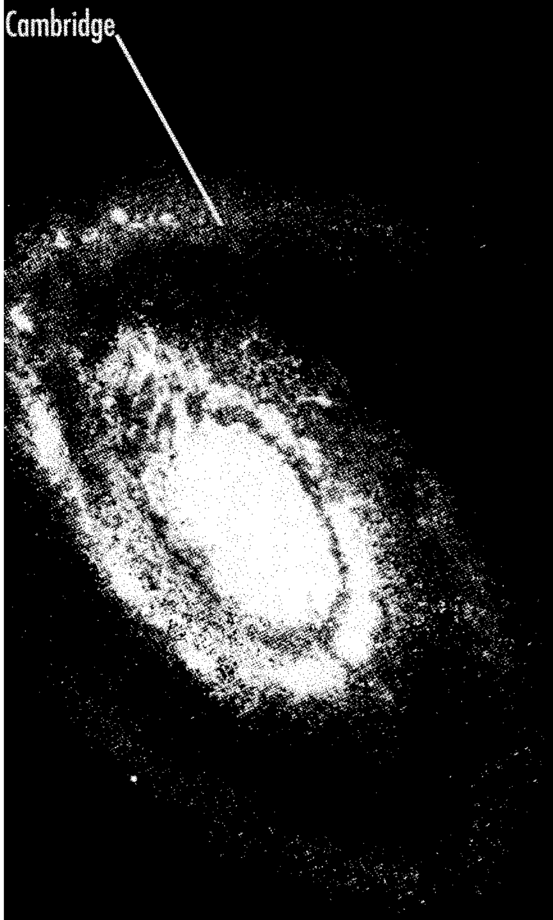

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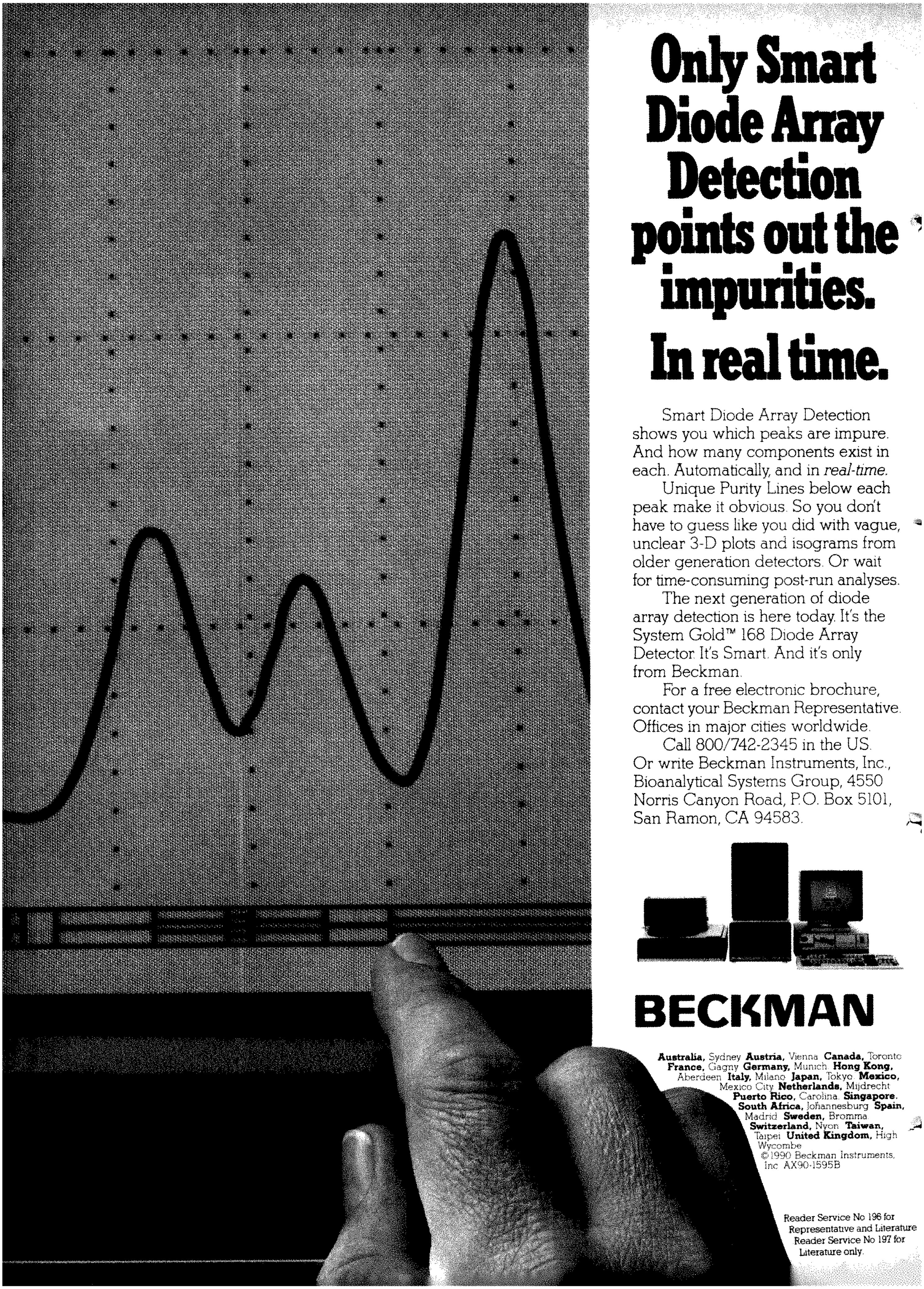

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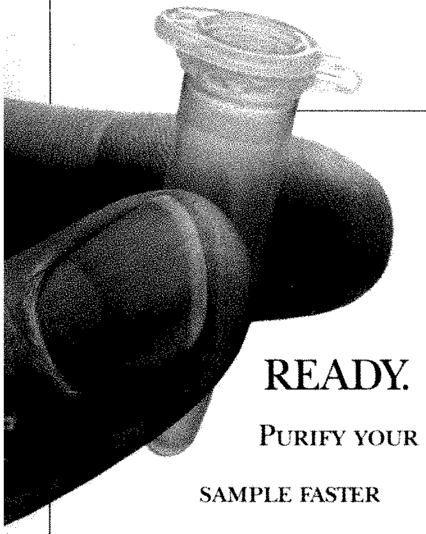

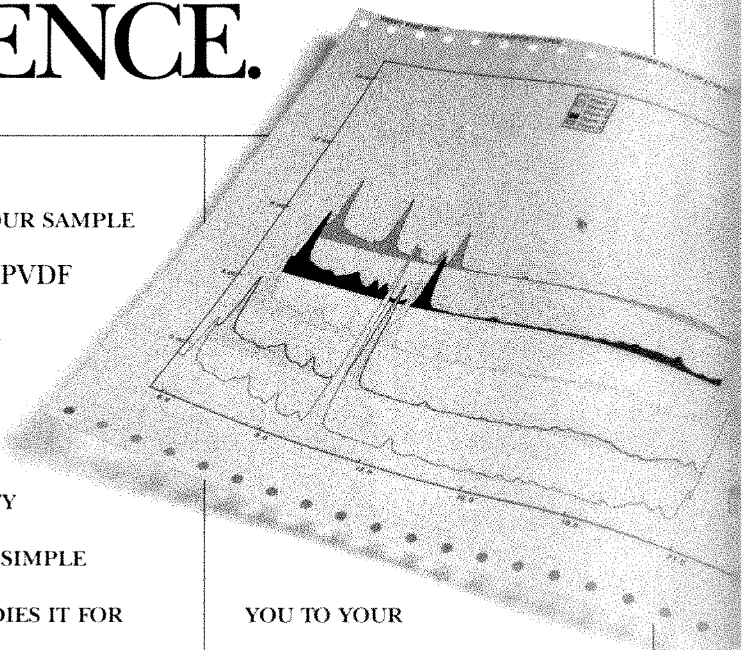

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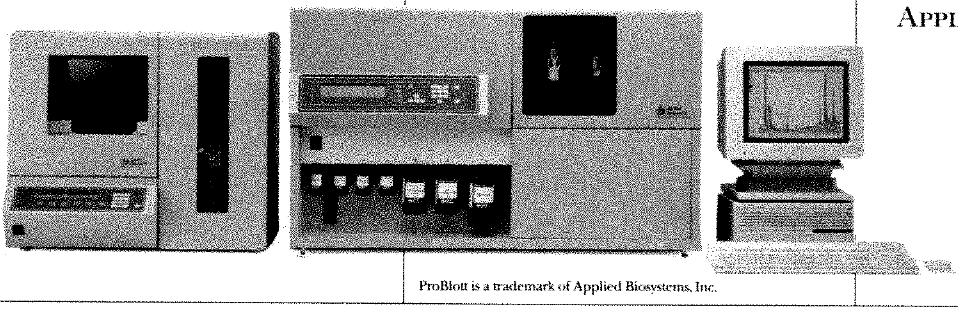

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What to pay for nuclear power?

A House of Commons committee has thrown new light on the ways in which British governments too often make bad decisions, especially where technical matters are concerned.

POOR (in both senses) Britain is paying a heavy price for the incompetence of its public administration, especially of technical matters. That is the chief message of the splendid report from the House of Commons Select Committee on Energy published last week (see also page 7). Ostensibly, the committee set out to tell why the nationalized electricity utility in England and Wales called the Central Electricity Generating Board (CEGB) should have told a public inquiry in 1987 that the price of electricity from a newly constructed pressurized water reactor (PWR) would be 3.09 pence per kilowatt-hour, and should have been quoting prices more than twice as much when negotiating with the government the terms on which it should be privatized. Readers of the report will not be better able to name a figure for the cost of nuclear electricity, but they will have a better understanding of the vacillations of government policy on nuclear energy.

It is a bizarre tale. Since 1972, the CEGB has been seeking to build PWRs, on the grounds that they could be built more quickly and would be intrinsically more economical than the gas-cooled reactors it had inherited from Britain's earlier flirtation with nuclear power. In 1979, the incoming Conservative government said it would sanction a programme to build ten PWRs over some forthcoming decade, but was then constrained by its predecessor's decision that none would be built until there had been a public inquiry. By the time the construction of the first reactor had begun in 1987, the government had also decided that the electricity industry should be "privatized". But it failed to appreciate that its nuclear policy and privatization policy might be in conflict. Now there is a gigantic muddle.

What the energy committee has most usefully done is to throw light on the casual ways in which these policies were made. The plans to privatize the electricity industry were made public in February 1988 by the then Secretary of State for Energy, Mr Cecil Parkinson, without a serious attempt to tell whether they were feasible. On the generating side, CEGB was to be split into two parts, 'Big G' and 'Little G' (now National Power and PowerGen respectively), but in the face of the CEGB's protests that nuclear power would be a problem.

So it has proved to be. First, in July last year, the government was forced to concede that the older gas-cooled reactors would have to remain in public ownership: reactors nearing the ends of their lives, but saddled with huge liabilities for decommissioning, would hardly have been an attractive investment. But then, last November, it had to go the whole hog, and lump all nuclear generating capacity into a third company, called Nuclear Electric, that will remain in public ownership.

The fascinating argument between the CEGB and the government, now revealed, about the price of nuclear electricity has been a proxy for the Department of Energy's failure to understand what privatization would entail. Should the discount rate (the notional cost of money) be 8 or 10 per cent? Is it surprising that CEGB, when told to ask British Nuclear Fuels (ironically, publicly owned) for a fixed price for reprocessing fuel, should be quoted a price 30 per cent greater than expected? Or that the CEGB should want to amortize new reactors over 20 years (rather than the full 40 years of life expected) to allow, in part, for the political risks ahead? In the end, the government took the view that the CEGB was asking for too much, and said no.

This sorry sequence of events may have permanently queered the pitch for nuclear power in Britain, and will also have played havoc with the public accounts. Nuclear Electric will operate existing reactors with the help of a subsidy collected from electricity consumers and called a Fossil Fuel Levy. (Britain will thus be the first industrialized country to have a carbon tax.) The Energy Committee is right to demand that the fixing of this charge should be transparent. Further nuclear construction has been postponed until a review in 1994. The Energy Committee is right to insist that the outcome of that review should be publicly debated before future policy is made. Meanwhile, there is a PWR half-built in Suffolk whose future is uncertain; why not finish the plant but not load it with fuel? That would avoid decommissioning costs and provide a lasting monument to four decades of mismanagement. ☐

Hubble trouble

The defects that have come to light in the Hubble Telescope are a serious setback for research.

THERE was always a risk that the Hubble Telescope, already more than three years late, would have been lost during the tricky process of putting it into orbit. That it should turn out to be incapable of being focused accurately is not much better (see page 3). It hardly matters that there will now be a grand inquisition to tell who is to blame, or that the US National Aeronautics and Space Administration (NASA) believes that an astronaut may be able to fix the trouble in 1993. The plain fact is that a decade's work by hundreds of skilled researchers has been undercut by an elementary error. Some hope that useful science can be salvaged, but three years out of an expected lifetime of fifteen are one loss, the inevitable degradation of many Hubble instruments another.

What should happen now is what matters most. Even if there were funds in the space science budgets of NASA and the European Space Agency (a 15 per cent partner in the venture), building another Hubble and relaunching it would not serve a useful purpose. The design of the instrument has its origins more than a decade back, while something else might in any case go wrong next time. Beginning now on the design of an even better instrument might mean that there was something to show for the effort early next century, but that is a long time to wait. By then, in any case, the design and construction of terrestrial telescopes will have reached the point at which only a radically better successor to Hubble would be worthwhile.

Last week's disappointment points sadly in only one direction — that Hubble loaded too many of the research community's eggs in the same basket, and a frail basket at that. Even if Hubble had been launched successfully, the intermittency of seeing caused by the Earth's shadow and the difficulties of planning the ponderous reorientation manoeuvres of the telescope to make the best use of its expected life, however splendidly accommodated by the software engineers, are a proof that an instrument as sophisticated as this deserves a much higher orbit. But that would mean extra power for data transmission, and an even more elaborate package . . . .

It will nevertheless be disastrous if this setback means that high-resolution astronomy from orbit is now abandoned, or postponed until after 1993. Low-resolution satellite astronomy in other than the visible regions of the spectrum has been a great success. The forthcoming ROSAT X-ray telescope will have greater resolution than its predecessors, though it has been much delayed. But there is an urgent need for complementing ground-based observations in the visible region with greater resolution from beyond the atmosphere. The need is for much simpler instruments in which versatility and optical speed are sacrificed for resolution and which are, perhaps, designed to answer predetermined questions. One virtue of instruments in orbit is that their users are not forced to hurry. They can sit there for days on end, collecting light from single objects. It would not be out of order that some imaginative agency should seek to lift the present gloom by running a competition for sufficiently robust designs. After recent experience (not only of Hubble) they should be capable of being launched by old-fashioned rockets, not through the bottleneck of the shuttle, which has now been thoroughly discredited. □

Himalayan clouds

The expedition to test Professor V. J. Gupta's claims is in doubt; Chandigarh should take other steps.

THE Panjab University of Chandigargh should not be too disconcerted that Professor V. J. Gupta has been taken ill and that the expedition he was to lead to some of the disputed fossil-sites in the Himalayas will probably not now set off in the next few weeks. Gupta's inability to travel has understandably prompted other members of the proposed expedition to say they will not go either. But the notion of testing the veracity of a whole corpus of Himalayan palaeontology by collecting fresh material from places and strata nominated by Gupta was never a particularly good one, anyway, at least without much more careful preparation than the proposed expedition had been given.

The essence of the doubt cast on much (not all) of Gupta's work is that some of the fossils he has described are unlikely to have come from the places in which he said he found them. Verification is complicated by the vagueness of the descriptions of locations in the published reports. (Sadly, Gupta is not the only palaeontologist to have erred in that respect.) A well-planned expedition would have begun with an attempt, independently of Gupta, to identify a few among the disputed fossils for which verification could be expected to be at once feasible, straightforward and in some sense clinching. Then the expedition would have known what it was looking for and, broadly speaking, where. It would not have been a particularly comradely expedition, of course; Gupta, ostensibly the leader, would have felt an uncomfortable pressure to deliver the goods under the eyes of watchful, even sceptical companions. But in the event, very little thought seems to have been given to the planning of the expedition except that it should set off some time this northern summer.

Dr R. P. Bambah, the vice-chancellor at Chandigarh, has rightly said that he wants neither a "witch-hunt" nor a "whitewash". No scientist whose work has been as seriously questioned as has been Gupta's deserves less of his university. But there are simpler procedures on which the university could and should fall back. By now, there have been such specific complaints against some of Gupta's conclusions that an expedition of verification need travel no further than his laboratory. Some of the disputes that have arisen could probably be settled if Gupta were asked to furnish a group of independent palaeontologists with original specimens (or to explain why they do not exist). The question is not whether there are, for example, conodonts in some Himalayan formations, but whether Gupta's past published reports of them are sustained by the evidence. Going off to the mountains is neither a necessary nor a sufficient test of that. What Chandigarh must recognize is that its own reputation will suffer from further delay. □

Hubble Telescope has misshapen mirror

■Non-imaging work to be emphasized ■Corrective lens by 1993?

Washington

The Hubble Space Telescope (HST), its builders have proudly claimed, contains the most precise optical system ever made, with reflecting surfaces polished to an accuracy of less than one-fiftieth the wavelength of light. That claim is still true.

But unfortunately, as officials of the National Aeronautics and Space Administration (NASA) admitted last week, the optical system was precisely made to the wrong shape, making HST's resolving power the same as that of the best ground-based telescopes, not ten times better.

But HST astronomers are confident that much of the science programme can be salvaged, and the defect is simple enough that replacement cameras equipped with corrective lenses, which could be installed by a space shuttle crew three years from now, will restore HST's full acuity.

The flaw was discovered as NASA engineers began to adjust the position of HST's secondary mirror, a small convex mirror that reflects light from the 2.4-m concave primary into the scientific instrument package. By shifting the secondary up and down the axis, astronomers expected a stellar image to come into perfect focus.

But the image could not be focused, and analysis of the way it changed as the secondary was moved indicated that HST suffers from spherical aberration caused by a departure of the radial profile of one of the mirrors from the required shape. The defect was such a "textbook example", according to deputy project manager Jean Oliver, that it must have been ground into the mirror during manufacture.

The departure of the optical surfaces from the required shape is small — about half a wavelength of light — but enough substantially to reduce the image quality (see below).

Most planned HST observations requiring high-resolution imaging will be put aside, but NASA officials were anxious, when announcing their predicament last week, to emphasize the "unique and important" science that HST can still do. Project scientist Ed Weiler pointed out that three of the HST instruments, the high-speed photometer, the high-resolution spectrograph and the faint object spectrograph, are non-imaging devices and had been expected to do much of their most significant work in ultraviolet regions of the spectrum, which are inaccessible from the ground.

The loss of resolution means that observations of faint, finely detailed objects will generally not be feasible, and HST managers will replace such observations with proposals that had been turned down for lack of available time.

Weiler, along with deputy project manager Jean Oliver, was hopeful that HST's spherical aberration can be so exactly diagnosed that it will be possible to design a simple corrective lens to restore HST's full ability. An improved Wide Field/Planetary Camera is in fact already partly built, and with corrective lens added could be installed during a scheduled shuttle flight as early as June 1993.

The same can in principle be done for the Faint Object Camera, but Peter Jakobsen, HST project scientist for the European Space Agency (ESA), which provided the camera, said that no specific plans could yet be made.

While astronomers were mostly interested in finding remedies, NASA is setting up a review panel chaired by Lew Allen, director of the Jet Propulsion Laboratory, to determine the cause of the defect. Oliver was adamant that HST's optical design was correct, and Bob O'Dell of Rice University, who chaired a space telescope planning group in the 1970s, agreed that the design had been checked by so many people that any possibility of a flaw had been eliminated. The error, Oliver concluded, must therefore have been in the mirror testing procedure.

The primary and secondary mirrors were never tested in combination. According to Oliver, a test of the optical system of HST would have required the construction of an optically flat surface as big as the primary; the cost would have run into hundreds of millions of dollars. Instead, the two mirrors were separately tested for conformity to the desired profile. Somehow, during the manufacture and testing, one of the mirrors was made to the wrong shape, and it will be the job of the review panel to discover how an error that should easily have been caught remained hidden until three weeks ago.

That job may not be easy. HST's mirrors were completed in 1981, and few of the people now involved in the project have any connection with those early days.

When NASA put construction of the HST optical system out for bids, the Perkin-Elmer company, an experienced builder of high quality optics, sent in a proposal that included a detailed account of how the optical surfaces would be separately finished to NASA's specifications. Although, as O'Dell recalls, some astronomers at the time would have been happier with an integrated test of the optics, Perkin-Elmer's proposal was judged adequate to the task.

Perkin-Elmer (now Hughes-Danbury after a take-over) won the contract and built the mirrors, but project managers at NASA's Marshall Space Flight Center (MSFC) in Huntsville, Alabama, had responsibility for ensuring that the terms of the contract were fully carried out. The review panel will reconstruct what happened both at MSFC and at Perkin-Elmer to find out how the faulty mirrors passed muster at both places. **David Lindley**

...and the good news

The optical system of the Hubble Space Telescope was designed to put about 70 per cent of the light from a point source into an image spot 0.1 of an arc second in diameter. Atmospheric turbulence prevents the best ground-based telescopes from producing images smaller than about one arc second.

A photograph made on 20 May by HST's Faint Object Camera (FOC) resolved stars into a central bright spot less than 0.1 arc second across and containing about 20 per cent of the light, the remainder being spread across a one arc second disc. It now turns out that FOC's first shot is as good as it will get.

At first, NASA officials and astronomers emphasised HST's capacity to do non-imaging photometry and spectroscopy, especially in the ultraviolet, where HST's chief advantage is simply to be above the Earth's atmosphere. But in the days following the announcement of the flaw, some have become more optimistic.

Peter Jakobsen of the European Space Agency, which built the FOC, says that because HST's images have a prominent core surrounded by a fainter halo, images of high-contrast objects, such as densely packed stars at the centre of globular cluster, will still be informative. And the fact that HST's distortion is of a constant, wavelength-independent form means some computer image-reconstruction techniques may be useful.

On the other hand, HST will not be able to see extended objects of low surface brightness any better than ground-based telescopes. It had been hoped, for example, that the FOC would be able to photograph the faint galaxies in which distant quasars are thought to reside, but Jakobsen now thinks the quasar light that spills into the one-arc-second halo will overwhelm any galactic light that might be there.

Nevertheless, Jakobsen thinks NASA was too "downbeat" in saying that virtually all the imaging work of HST will be useless, and emphasized that it is "early days" for astronomers thinking of ways to compensate for HST's deficiency. **D.L.**

COMETARY PROBE

Giotto steals a ride

Munich

THE Earth lost a minute fraction of its orbital velocity earlier this week as the European Space Agency (ESA) Giotto space probe stole a little of the Earth's energy to help it on its way to a rendezvous with the comet Grigg Skjellerup. It was the first time the manoeuvre, the same "gravity assist" that helped the NASA Voyager probe on its journey from Jupiter out to Saturn and Neptune, had been executed using the gravitation field of Earth. According to ESA researcher Trevor Morley, Giotto will gain 3.1 km/sec in velocity, meaning that the Earth will slow in its orbit by one millimetre in every million centuries.

A free ride on the Earth's gravity was a welcome break for a project that is beset by funding uncertainties. Despite the tremendous success of the original Giotto mission to Comet Halley in 1986 (see *Nature* **320**, 202 & **321**, 259; 1986) and the potential for further cometary exploration, ESA voted last month to make the Giotto Extended Mission (GEM) an optional, not mandatory, project within its science programme. Member states will meet in September to discuss whether they are prepared to pay for Giotto.

Project manager Manfred Grensemann at the ESA ground station in Darmstadt is "optimistic" that enough support can be found. He estimates that the next phase of the mission would cost 10 million ECU (about $8 million), mostly for the communications needed to keep in touch with the probe as it moves farther away.

"Compared to starting a new mission, this is a bargain", says GEM project scientist Gerhard Schwehm. Schwehm said that the second flyby would be a unique opportunity to expand knowledge of comets. It will be especially interesting, he said, to study the interaction of the coma of the comet and the solar wind, as well as to compare the distribution and optical properties of dust around Comet Grigg Skjellerup with that around Comet Halley, which had at least 100 times more of it.

Giotto was one of five spacecraft to approach Comet Halley in 1986, but it moved far closer than the others and came away with dazzling photographs of the nucleus. After the Halley encounter, Giotto was put into 'hibernation' to conserve power as researchers sought a suitable second comet to study with the craft. The probe will now be put into hibernation again until May 1992, when it will 'awaken' and prepare for the next encounter.

Unfortunately, said Grensemann, the camera that took the dramatic shots of the nucleus of Comet Halley is no longer operating — the sensors still function but no light reaches the aperture. But seven other instruments on board are at least partly operational and Grensemann expects them to deliver good data.

The researchers chose Comet Grigg Skjellerup as Giottos's target as much for practical as for scientific reasons. The comet was in a position in its orbit around the Sun that meant only minor adjustments in the trajectory of Giotto were needed for them to meet.

Steven Dickman

RHEUMATOID ARTHRITIS

New institute for Berlin

Munich

A RESTORED villa on the Kleiner Wannsee, a lake within West Berlin city limits, will become home for a new institute for research into the causes of rheumatoid arthritis. Immunologist Avrion Mitchison of University College, London, has been named as scientific director and will work in Berlin half-time.

As a result of a political squabble over the institute between the left-wing coalition of Social Democrats and Greens which took office last year in West Berlin and the Conservative Christian Democrats who begin the project, the institute will study not only the molecular biology of the disease but will also look at "psychosocial factors" that contribute to its development. Mitchison says that this area has been neglected in rheumatoid arthritis, especially in comparison to the attention it has received in cancer.

Steven Dickman

CONFERENCES

Changes at Dahlem

Munich

SILKE Bernhard, scientific director of the Dahlem Conferences, is to step down because of ill health. A physician by training, Bernhard had been credited by participants with building the high international reputation of the conferences.

Last year, Bernhard struggled to maintain the conferences when their original backer, the 'Donors Association for Promoting Arts and Sciences in West Germany' (*Stifterverband für die Deutsche Wissenschaft*) withdrew its support. The Free University of Berlin took over sponsorship in November (see *Nature* **340**, 86 & **342**, 466; 1989).

Jennifer Altman, a neuroscientist and formerly on the staff of *Nature*, will act as interim scientific director until an international search can be arranged for a permanent director.

Steven Dickman

JAPANESE UNIVERSITIES

Murmurs of complaint

Washington

JAPAN's national university professors are all employees of the government, which puts them in an odd position when they want to protest to the government about university conditions. But last month, the Association of National Universities finally succeeded, after years of trying, in winning funds from the Ministry of Education, Culture and Science to set up a committee to study their own financial difficulties.

The association represents all the 93 national universities directly supported by the government and has responsibility for setting up the general entrance examination.

Akito Arima, president of the association and of the University of Tokyo, recently described the universities' difficulties in a magazine of the Ministry of Finance. "In Japan about 1 per cent of GNP is allocated to college education and research, but in the United States it is 1.5 per cent. Within the limited budget, priority is placed much more on building new laboratories, moving universities, and big science, and less on the reconstruction or the maintenance of existing laboratories.

"As a result, buildings and equipment are becoming old and dilapidated. Good research cannot be produced by laboratories that are too small and lack both good equipment and money. We must find other financial sources." The new committee has not decided its plan of action and will first gather basic data.

Shigeko Segawa

ANTARCTICA

Airstrip plans on ice

Sydney

AUSTRALIA has been forced to abandon plans to build an 'environmentally friendly' A$1 million airstrip close to its Casey Base in Antarctica. The experimental runway was built on ice to minimize the effect on wildlife, most of which lives on the very small part of the Antarctic that is rock. Construction of a rock runway at the French Antarctic base led to an international outcry as Greenpeace claimed that an important breeding ground for Adélie penguins had been destroyed.

But ice has not proved to be safe after all, according to a report by the Royal Australian Air Force. Last summer, the report says, a vehicle broke through the ice on the runway and sank into the water beneath. Because of the risk of the runway melting and breaking up, the air force argues that a gravel airstrip should be built.

The Australian Department of the Environment's Antarctic Division has proposed an alternative site for an ice and snow runway 30 km away from Casey where the climate is harsher. The United States has a successful ice strip at its McMurdo base south of Casey.

Tania Ewing

CHEMICAL WEAPONS

First test of incineration

Boston

IF all goes as planned this week, the US Army will destroy by incineration a first batch of chemical weapons at its new facility on remote Johnston Island in the Pacific Ocean. The trial incineration, which the Army calls "operational verification testing", is the first step in fulfilling the agreement between US President George Bush and Soviet President Mikhail Gorbachev earlier this year to destroy the bulk of the superpowers' vast chemical arsenals (see *Nature* **345**, 192; 1990).

Although the chemical weapons agreement was widely welcomed, both sides acknowledge that destroying the weapons presents environmental dangers that could engender considerable political opposition. On the US side, the Johnston Island facility represents the US Army's attempt to show that the ageing chemical weapons can be disposed of safely. The incinerator was completed last year, before the recent agreements, to destroy a large stockpile of chemical weapons that had been stationed in the Pacific during the Second World War. These weapons, amounting to several thousand tons, have been stored since 1971 on Johnston Island, which is some 700 miles from its nearest Hawaiian neighbour.

Army chemists say that because the chemical components of the nerve, mustard and blister agents to be burned are known so precisely, and because of the facility's state-of-the-art design, it should generate fewer toxic emissions than conventional trash incinerators. But this optimistic view is not universal. Dozens of environmental groups have objected to the facility during the lengthy political process required for it to open, decrying the potential air pollution and toxic solid wastes generated by incineration and favouring alternative disposal methods.

Even as testing begins, Sebia Hawkins of Greenpeace says that there is "still hope that the facility can be stopped". But environmentalists concede that the Army has largely sidestepped opposition because the island is so remote and lacks a native population. Future incinerators to handle the vast bulk of the nation's stockpiles remain to be built, and are likely to generate far more political opposition.

Almost everyone involved in the project admits that a deadly accident could occur. As carefully designed as the handling procedures are, the sheer scale of the operation is worrying.

The Johnston Island facility will handle thousands of tons of lethal chemicals — some of which can kill in microgram quantities — and burning all the chemical agents now stored on the island is expected to keep the incinerator running steadily for over a year.

Adding to the controversy is the fact that later this year another 430 tons of chemical weapons are scheduled to arrive at the island. The new intake comes from West Germany in the wake of an agreement in 1987 between then US President Ronald Reagan and German Chancellor Helmut Kohl that the United States would remove its chemical stockpile from German soil. The agreement stipulates that the weapons must be removed by the end of this year, but does not specify where they should go. Johnston Island is virtually the only place in the world that can handle them, so the plan is that the weapons will be moved by truck, rail and ship, under tight security, from Clausen, Germany, to Johnston Island.

All told, the Johnston Island facility is marked for the incineration of less than 7 per cent of the US stockpile. The current plan calls for the other 93 per cent to be incinerated in the continental United States, at military facilities in Alabama, Arkansas, Colorado, Indiana, Kentucky, Maryland, Oregon, and Utah. Public opposition is already mounting.

Meanwhile, the Soviet chemical weapons stockpile is said to be even larger than that of the United States, and the Soviet destruction programme is in its infancy. The Soviet president has asked for US help with the Soviet effort to destroy chemical weapons, and collaboration is written into the formal agreement between the two countries. Elisa Harris, an expert on chemical and biological weapons at the Brookings Institution in Washington, says that the Soviet population has even less confidence in its government's ability to do the job than Americans have in the US chemical weapon destruction programme.

She calls the domestic Soviet problem part of a "post-Chernobyl syndrome". The political controversy generated in both countries is likely to make Johnston Island an important model for both sides. US experts have met already with Soviets in Geneva and Moscow to discuss technical issues of chemical weapon destruction, and a Soviet delegation is expected soon to visit a small-scale prototype US facility constructed several years ago at an Army base in Tooele, Utah. As Harris puts it, "the Soviets are clearly capable of destroying their own chemical weapons", but with the domestic political difficulties involved in carrying out such programmes in either country "it will be interesting to see just how much cooperation each side has in mind".

Seth Shulman

CONSERVATION

Tigret spells hope for endangered species

Washington

THE world's first test-tube tiger was put on display at the National Zoo in Washington DC last week. Although the cub was born two months ago, zoo officials have up until now not had the confidence to show it to the public. Two other cubs born at the same time died in their first weeks.

Jeff Tinsley, Smithsonian Institution

Test-tube baby — the world's first IVF tiger.

The cub, named Mary Alice, represents a $2 million dollar investment in *in vitro* fertilization (IVF) technology which the zoo hopes will help to preserve endangered species. Mary Alice is a Siberian tiger, a rare subspecies of tiger of which only 200 remain in the wild. Seven hundred are kept in zoos around the world, which means that captive breeding provides the best chance of saving it from extinction.

The IVF technique is likely to be of particular help because it allows sperm from a valuable male in one country to be used to fertilize eggs from a partner in another country, without all the difficulties of transferring the animals themselves.

Since 1984, the National Zoo, in co-operation with Henry Doorly Zoo in Omaha and the Minnesota Zoo, has being working to adapt IVF techniques originally developed for humans. After IVF was used successfully with domestic cats, the technique was tried on the Siberian tiger. Three test-tube cubs were born in Henry Doorly Zoo on 27 April. A mobile IVF laboratory developed by the National Zoo and taken to Omaha for the placement of the three fertilized eggs in the surrogate mother tiger will provide artificial breeding technology to other zoos in the future.

Shigeko Segawa

MONTREAL PROTOCOL

Warming to global agreement

London

UK Environment Secretary Chris Patten hailed last week's Montreal Protocol meeting in London as "a major step forward in environmental diplomacy", after participants agreed to phase out ozone-depleting chemicals more quickly than agreed at Montreal in 1987 and both the Indian and Chinese delegations said they would urge their governments to join the protocol.

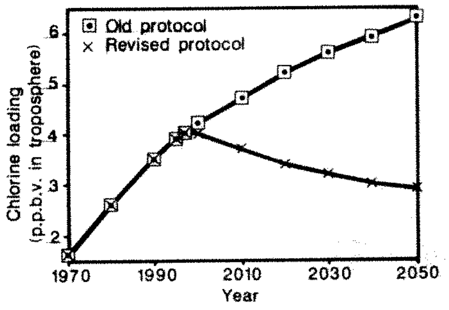
The involvement of India and China, which both have the potential to develop large chlorofluorocarbon (CFC) industries, was in doubt until the United States withdrew demands that it should have control over an international fund to help developing countries finance alternatives to CFCs, in proportion to its 25 per cent financial contribution.

Under the 1987 Montreal Protocol, CFC use was to be halved by 1998, and halon use was to be frozen at 1986 levels in 1992. The new protocol has CFCs phased out altogether by 2000, following a 50 per cent cut by 1995 and an 85 per cent cut by 1997. Halons are also to be eliminated by 2000, except for some essential firefighting uses, following a 50 per cent cut in 1995.

Carbon tetrachloride and methyl chloroform, not covered by the 1987 protocol, are also to be phased out, by 2000 and 2005 respectively. Although these chemicals damage ozone less than CFCs and halons, they are used widely in industry.

Many nations pushed for even quicker progress, and near the conference's end, a 13-nation breakaway group led by Finland announced that its members would end their use of CFCs by 1997, irrespective of the final form of the protocol. US, Japanese and Soviet opposition prevented the conference adopting the 1997 target date, although the target will be reviewed again in 1992. Patten said that the United Kingdom, while not among the 13, would aim to eliminate CFC use by 1997, except for medical uses where alternatives are not yet available.

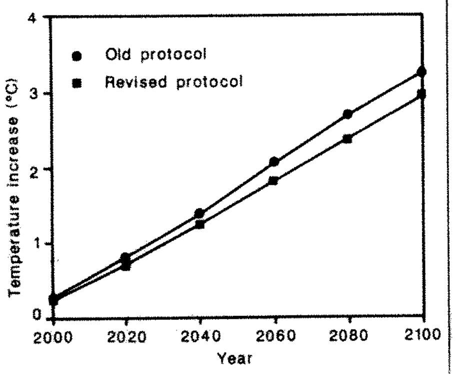
Scientists at the UK Department of the Environment have produced a preliminary analysis of the effects of the new protocol on atmospheric chlorine loading (see figure). By 2050, chlorine loading in the troposphere, at 2.9 parts per billion by volume (p.p.b.v.), will be less than half that predicted under the 1987 protocol. Chlorine loading in the stratosphere, and hence effects on ozone depletion, will lag behind this by about five years.



Joe Farman, a member of the UK Stratospheric Ozone Review Group and the discoverer of the Antarctic ozone hole, is worried that even the new controls do not go far enough. He says that the aim for the London meeting should have been to reduce atmospheric chlorine loading back to about 2 p.p.b.v. as quickly as possible. The new protocol will not approach this target until late next century, after concentrations rise to an unprecedented 4 p.p.b.v., a timetable Farman describes as "madness".

Peter Aldhous

Effects of new protocol



New controls on ozone-depleting chemicals outlined in the revised Montreal Protocol will also reduce global warming, as some of the chemicals involved are greenhouse gases. The figure shows the likely effects of the new protocol on increase in global mean temperature from the 1990 value over the next century, carried out for *Nature* by Professor Tom Wigley of the University of East Anglia.

The prediction is from STUGE (Sea level and Temperature change Under the Greenhouse Effect), an interactive microcomputer-based model developed by Wigley and his colleagues Tom Holt and Sarah Raper. STUGE incorporates models used by the Intergovernmental Panel on Climate Change working group I, and Wigley makes the key assumption that a doubling of atmospheric carbon dioxide will increase temperature by 2.5 °C.

Wigley says that his prediction includes some *ad hoc* judgements about the use of the hydrochlorofluorocarbons that are expected to replace CFCs in the short term, and stresses that the model gives a best estimate, around which there may be some margin of error.

The amended Montreal Protocol has only a small effect on global warming, reducing temperature increase between today and 2100 by only about 0.3 °C. But Wigley says: "if we're going to make any progress, it will be the sum total of small effects". **P.A.**

BIOMEDICAL RESEARCH

Young researchers go short

Washington

THE hard times being faced by young biomedical researchers in the United States were the major focus of a meeting on 'Support for Biomedical Research' held last week under the sponsorship of the National Academy of Sciences and the Institute of Medicine.

Although the National Institutes of Health (NIH)'s total funding has increased moderately over the past few years, there has been a steady fall in the percentage of successful applications in the 'new and competing' grants category — the grants that launch a young researcher on an independent career. This year as few as 22 per cent of applications are likely to be successful, down from almost double that percentage three years ago.

Many scientists now think that the problem of a shortage of funds was compounded by a decision made in 1985 by James Wyngaarden, then director of NIH, to increase the length of time for which grants run. The decision was made, in part, to provide scientists with greater financial stability and to relieve them of the inordinate amount of time spent renewing grants. But it has had the unintended side effect of intensifying competition among scientists fighting for their first grants.

Although most scientists agreed that the fundamental problem was a lack of money, David Baltimore, president of Rockefeller University, questioned whether available money was being spent efficiently. He claimed that the Howard Hughes Medical Institute, a nonprofit organization, will win a "disproportionate result" from spending just $44 million this year to support young scientists.

Industry is continuing to play a large role in biomedical research. In 1979, industry funded 29 per cent of health research and development. Last year, this had risen to 45 per cent. As industry leaders acknowledge their debt to public-sector research, Baltimore suggested that industry could do still more to help by contributing about $100 million a year to support young researchers and infrastructure development. But Senator Dale Bumper (Democrat, Arkansas), who serves on the committee that appropriates funds for NIH, said that the federal government "must take a strong lead in basic research" and not leave it to *ad hoc* corporate decisions".

Diane Gershon

UK NUCLEAR POWER

Pressurized accounting

London

THE Department of Energy, former Energy Secretary Cecil Parkinson and the now defunct Central Electricity Generating Board (CEGB) were all at fault in the abortive attempt to privatize Britain's nuclear power stations. That is the conclusion of an all-party House of Commons committee which has investigated the spiralling cost estimates for nuclear-generated electricity in the run-up to privatization of the electricity industry last year.

Michael Clark, a Conservative Member of Parliament who chairs the energy select committee, said that these costs more than doubled from CEGB estimates given in 1988. The increases forced the government last November to retain Britain's nuclear stations in public ownership, and to freeze plans to build three more pressurized water reactors (PWRs) besides that now being built at Sizewell B, in Suffolk (see *Nature* **342**, 213; 1989).

The report acknowledges that some of the cost increases, for example those due to tightened environmental safety standards, were genuine. But it suggests that the bulk of the increases reflect the fact that the CEGB had never previously provided adequate costings for nuclear generation. The report concludes that "there has been a systematic bias in CEGB costings in favour of nuclear power". Only impending transfer to the private sector forced the CEGB to account for realistically low-risk investment and high returns on capital. After being told for years that nuclear generation was cheaper than other forms of power, it is now "close to the truth to say that nuclear electricity is expensive", Clark said.

The committee censures the Department of Energy and successive governments for failing to obtain realistic nuclear costings from the CEGB. Parkinson is attacked as the minister responsible for privatization, in particular for ignoring the energy committee's advice in July 1988 — which doubted the wisdom of privatizing nuclear power and suggested the government had "glossed over" nuclear costs. But two Conservative members of the committee, Malcolm Moss and Michael Stern, distanced themselves from the attack on Parkinson at the report's launch, because the former Energy Secretary had not been able to give evidence to the committee. An earlier leaked draft of the report had been more forthright in its criticism of Parkinson, saying he had acted like a "dilettante".

The energy committee's report followed close on the heels of a rise in estimates of the cost of completing the Sizewell B PWR caused by contraction of the PWR programme. Costs that would have been shared across the four planned stations will now be borne by Sizewell B alone. The revised capital cost of Sizewell B, at £2,030 million in 1987 prices, is more than £300 million greater than the original 1987 estimate.

The environmental group Friends of the Earth (FoE), which leaked the figures last week, and opposition Labour energy spokesman Frank Dobson have called for Sizewell B to be cancelled. But John Collier, chairman of Nuclear Electric, the government-owned company that now runs the old CEGB's nuclear stations, said that this would be uneconomic, and dismissed FoE's claim that construction work is running behind schedule. Collier said Sizewell B's costs are not "largely out of control", as FoE had claimed: all the revisions were due to the cancellation of the three further PWRs.

Sam Goddard, director of construction and future programmes with Nuclear Electric, said that the cost of electricity from Sizewell B would need to be about 2.5p per kilowatt-hour in order to recover future investment that could be avoided if the project was cancelled. This is similar to the cost of electricity from a new combined-cycle gas turbine plant and less than electricity from a coal-fired station (between 3.3 and 4p per kilowatt-hour). But, if both future and past investment in Sizewell B is used to calculate costs, its electricity is more expensive than either gas- or coal-fired stations.

Clark said he believed that completion of Sizewell B was still justified. Apart from financial considerations, retaining a PWR may be useful in keeping open the option for a later expansion of UK nuclear power. This may prove necessary if 'carbon taxes' are levied on fossil fuels, or enforceable limits on carbon dioxide emissions are introduced, to counter the problem of global warming. **Peter Aldhous**

CHINESE DISSIDENT

Fang gets down to work

London

IN his first public appearance since his surprise release from China, dissident astrophysicist Professor Fang Lizhi made a short statement at the Royal Society in London last Friday. Fang looked forward to "a period of peace and quiet", in which to pursue his research, and declined to answer any political questions.

Associated Press

Fang Lizhi (left) is greeted by Sir George Porter, president of the Royal Society.

Fang has now taken up his position at the University of Cambridge's Institute of Astronomy, where he will continue his work in cosmology and large-scale structure of the Universe. Professor Martin Rees, director of the institute, says he is particularly pleased to welcome Fang, who had been extremely effective in promoting academic contacts between China and the West during the early 1980s.

The Royal Society will support Fang's research for up to a year, but Fang may eventually choose to settle in the United States. Peter Aldhous

SCIENTIFIC MISCONDUCT

Lawsuit problems

Washington

IN recent months, at least three unexpected lawsuits have introduced a wild card to the investigation of scientific misconduct. In each case, a whistle-blower has filed suit — claiming scientific misconduct — under an obscure provision of federal law that allows informants to sue for damages on behalf of the United States and to keep 30 per cent of the damages should they win. Known as a *qui tam* action, such a lawsuit is decided by a jury in federal court, effectively bypassing the scientific misconduct investigation process of the federal science agencies.

The law was designed to encourage whistle-blowing, especially in areas prone to fraud and embezzlement such as defence contracting.

But *qui tam* suits can be quite lucrative in scientific misconduct cases as well. Damages are trebled by law, and if a large National Institutes of Health grant must be repaid times three, a 30 per cent share could amount to many tens of thousand dollars. "Whistle-blowing for fun and profit", is how Crowell and Moring attorney Robert Charrow describes it.

Federal scientific integrity officials and congressional staff are watching the trend closely. "It's a real problem — the purpose of misconduct investigations is not to make anyone rich", says Greg Simon, staff director for the investigations and oversight subcommittee of the House of Representatives Science and Technology Committee. If *qui tam* suits continue, Congress may be forced to try to introduce legislation exempting scientific misconduct from the law, he says.

G. Christopher Anderson

MIT

Charles Vest is new president

Boston

After several false starts and a renewed extended search, the Massachusetts Institute of Technology (MIT) has officially chosen its new president. Charles M. Vest, a 48-year old mechanical engineer who is now provost of the University of Michigan, has accepted the job and been formally approved by the MIT Corporation, and is due to begin as MIT's fifteenth president in mid-October this year. At his first news conference at MIT, Vest bemoaned the state of US competitiveness, saying that "large segments of the nation have lost the will to excel". He pledged a "fundamental reassessment of engineering education".

Several leading contenders removed themselves from consideration, and one, biologist Phillip Sharp, declined the job a week after his selection was formally announced. Vest said he is "proud to be a colleague" of someone like Sharp who puts his research above his desire to be an administrator, and brushed aside comments about the long, tumultuous search.

Seth Shulman

JAPANESE SCIENCE

Land of rising publication

Washington

In the past decade, Japanese researchers have increased their representation in the pages of leading international science journals by over 50 per cent, according to a new survey in *Science Watch*, a newsletter published by the Institute for Scientific Information.

Organic chemistry leads the list of improved Japanese research fields, as measured by journal publication. The five leading chemistry journals recorded an increase in Japanese papers from 8.8 per cent in the first half of the decade to 11.3 per cent in the second half. Applied physics research, especially in the fields of solid state and condensed-matter physics, has been another strong area for Japanese scientists in the 1980s. Representation in top physics journals jumped from 5.1 per cent to 8.5 per cent over the decade.

Biology is the third area in which Japan has shown marked improvement in journal publication. Representation in the five leading biology journals increased from 3.3 per cent to 5.9 per cent in the past decade. But in the two leading multidisciplinary journals, *Science* and *Nature*, Japanese representation has grown only slightly. *Nature* continues to publish more than twice as many Japanese papers as *Science*: the ratio is now 1.8 per cent to 0.8 per cent, as compared to 1.6 per cent to 0.7 per cent a decade ago.

G. Christopher Anderson

UNIFYING GERMAN RESEARCH

Problems of streamlining

Munich

With the steamroller of German unification fast approaching, the East German Academy of Sciences last week began the awesome task of stripping away 14,000 of 24,000 jobs in order to create a streamlined core that can be integrated into West German science. Pressure from both the recent currency union and shrinking funds for science forced the East German government to give the academy only until the end of the year to complete its changes. Research institute directors did not learn of this decision until a meeting at the Adlershof research campus in East Berlin on 25 June.

Even the planned reduction will probably not be enough to satisfy the West Germans who will hold the purse-strings for science in a unified Germany. The number of employees who survive the academy cuts is likely to be under 10,000 — but even the renowned Max Planck Gesellschaft of West Germany has just 13,000 employees. That means that an academy with 10,000 employees may still seem excessive when the two German states are unified politically, a step now likely to take place in December. The academy will lose nearly half its budget as the industrial *Kombinate*, which provided support for contract research, disappear in the face of West German competition.

The East German Research Ministry, which is responsible for the academy in the current East German government, has promised to do its best, but is unlikely to be able to take up all the slack as funds disappear. East German Research Minister Frank Terpe said just two weeks ago that he would try to provide a two-year 'moratorium' on the closing of academy institutes to allow time for restructuring and for the influential West German science advisory council (Wissenschaftsrat) to survey academy research projects and recommend changes.

But this timetable is proving too slow for the West German Research Ministry (BMFT) which will take responsibility for the academy in a unified Germany. A BMFT spokesman says that the ministry cannot wait the 18 months requested by the Wissenschaftsrat to see which institutes and groups should survive, but would prefer a "rough assessment" to be completed by December. Though this is "not a perfect solution", he said, "we will try to push in this direction. No matter how much is required for the evaluation", he added, "money will be no obstacle." Wissenschaftsrat will have practical problems carrying out the evaluation so quickly. "It will be hard to get the experts in on short notice," warned Wissenschaftsrat chairman Dieter Simon.

West German Research Minister Heinz Riesenhuber will meet Terpe, East German Education Minister Hans Joachim Meyer and the heads of West German science organizations this week in order to try to force the pace.

The new East German scientific leaders have additional worries. Benno Parthier, just appointed director of an academy research institute in Halle and president-elect of the Akademie Leopoldina, said that such a quick change would not leave time for a "differentiated look" at what East German science could do.

Horst Klinkmann, a medical researcher in Rostock who last week became the first democratically elected president of the academy, said that if there is not enough time to adapt, it will leave a "scientific vacuum" in East Germany that could destroy the economy.

BMFT wants different institutes to receive different treatment. Some would be shut down or sold to industry — these would be easy to identify, according to a BMFT spokesman. Others will be maintained, after excess has been pared away, as institutes for applied research under the Fraunhofer Gesellschaft or perhaps even as part of the Large Research Establishments (*Grossforschungseinrichtungen*). There will be plenty of painful choices, especially within institutes that have to be reduced in size. "I have to consider the existence of the institute first, before the jobs of individual employees," said Parthier, who is giving up his summer holiday to begin the task. "But I have known these people for many years, and it is not their fault that they are in this position."

East Germans who had hoped for salvation through the Max Planck Gesellschaft (MPG) will be sorely disappointed by the announcement at the MPG annual meeting in Lübeck on 20 June that East German institutes would not be absorbed. Incoming MPG president Hans Zacher said the dialectical approach to science in East Germany had "turned on its head" the successful West German approach (which originated in the nineteenth century under the Reich) allowing freedom of research with generous state and industry support. "Trying to unify the two systems could destroy them both", he warned. "There is a lot of useful research in East Germany. . . Our problem is how to integrate it into a system based on freedom without changing ourselves." Simon, who is also director of a Max Planck Institute, considers such fears unreasonable. "We will not be 'infected' by the East German mentality, as some are supposing", he added. "Instead", he said, "we have to develop a 'we' mentality" together with the East Germans.

Steven Dickman

SCIENTIFIC MISCONDUCT

Investigators investigated

Washington

A year after it was first created, the National Institutes of Health's Office of Scientific Integrity (OSI) is still getting an on-the-job lesson in the difficult art of investigating scientific misconduct. Faced with a growing case load (nearly 80 at the last count), congressional and media scrutiny and criticism from universities and scientists who have passed through its doors, OSI is under pressure to prove in its second year that it can deal effectively with misconduct without stifling research.

The first step it will take is to publish, in the coming weeks, a set of formal procedures describing how OSI works. Included will be rules that will give those found guilty of misconduct a chance to negotiate their own sanctions and those found innocent a greater opportunity to clear their names. Congress is also considering taking matters into its own hands. A draft bill now being circulated by the subcommittee on investigations and oversight of the House of Representatives Science Space and Technology Committee would set new guidelines to assure fairness in university misconduct investigations and increase the protection of whistle-blowers.

Legal rights?

The lack of any published procedures has fuelled the principal complaint against OSI that it fails to accord basic legal rights to people accused of misconduct. And, because no one knows what action is being taken, OSI appears to take far too long to complete an investigation — the inquiry into the case of the *Cell* paper written by David Baltimore and others has now been running for over two years.

Another celebrated case, that of AIDS researcher Robert Gallo, raised new criticisms when OSI decided to appoint a National Academy of Sciences panel to oversee its investigation. If an oversight panel was needed in this case, why not in others? "It would appear that the entire *ad hoc* 'process' has been devised with at least one purpose in mind — protecting NIH from congressional criticism", wrote former Health and Human Service (HHS) deputy counsel Robert Charrow in last month's *Journal of NIH Research*.

The most severe criticism of the NIH investigatory process comes from those who have suffered at its hands. "Potentially career-destroying investigations should not be carried out the way mine was", says David Bridges, a Purdue University vision researcher who was found guilty of plagiarism by an NIH investigation last year (see *Nature* **340**, 173; 1989). In the three years since allegations were first made in his case, Bridges says he has spent uncounted hours and well over $20,000 of his own money on his defence. He still protests his innocence and is the only researcher to have gone through every appeal process up to and including a just-completed four-week debarment hearing.

Bridges must now wait until the end of the year to learn if, as a result of the hearing, the Department of Health and Human Services (HHS) will reverse the earlier NIH decision or concur and ban him from receiving federal grants. Until the debarment hearing earlier this month, Bridges claims that neither he nor his lawyers had been allowed to cross-examine witnesses or take testimony on the record.

Under the current NIH rules, a researcher "can be debarred without even getting the chance to testify", complains Bridges' lawyer, Houston attorney Leslie Ribnik. Debarment hearings are granted at the discretion of HHS. There are no laws that compel NIH to call the accused researcher to give evidence during earlier investigations, Ribnik claims.

University regulation

Although OSI has operated without making its own procedures public, it has laid out a set of rules for how universities receiving HHS money should conduct their misconduct hearings.

Once alerted to the possibility of misconduct, the university is expected to put together a panel to review the charges in no more than 60 days. If the panel finds cause for a formal investigation, the university is to conduct an investigation within 120 days. At the investigation stage, the university must inform NIH and submit a report on the outcome to OSI. If it is dissatisfied it may then carry out its own investigation.

Although institutional guidelines have been in print for over a year, there are still doubts over whether they are fair, in the sense of whether they grant the accused adequate rights of "due process".

One problem is that at no time is an accused researcher guaranteed the right to see the evidence or hear the charges against him or her. "You can spend a lot of money trying to respond to unnamed people and uncharacterized charges. Meanwhile, your funding can be halted and your grant application can be denied", says Barbara Mishkin, an attorney with the Washington law firm of Hogan and Hartson.

But Suzanne Hadley, OSI's acting director for most of its first year, says that granting full access to evidence and written transcripts has become OSI policy, even if they are nowhere published. Another unwritten OSI policy is to spell out the charges, although OSI sometimes draws the line at revealing the informant. "We try to depersonalize things — to treat it as a federal office making the accusations. Too often the whistle blowers feel that they're carrying the whole fight on their own shoulders", she says.

The draft congressional bill provides for even greater whistle-blower protection but its authors are wary of going too far. Greg Simon, staff director of the oversight and investigations subcommittee, is concerned that overprotection of informants could create "a new tenure track". If whistle-blowers are made sacred, universities may find themselves unable to dismiss them for legitimate reasons years later, he says. Meanwhile, both OSI and NIH as a whole are considering sanctions against those who make false accusations.

Due process

Simon's bill proposes more emphasis on due process, at least at the university level. For researchers under university investigation, the bill lays out a series of rights, including the right to an attorney, to call and cross-examine witnesses, to present evidence and to file a statement. In return, for universities that accept the guidelines, the bill offers protection against lawsuits by accused researchers.

Full due process within NIH investigations is a stickier issue. Hadley argues that it is inappropriate for OSI. "What we do is not a trial", she says. But others point out that because OSI reports directly to the Office of Scientific Integrity Review, the overseeing body within HHS that recommends sanctions, the combination has the effective force of a judge and jury. "A judicial procedure has due process, an investigation doesn't. "Which is OSI?" asks Charrow.

Inside NIH, criticism of the investigation process has been fanned by messy cases that last years and draw congressional charges of bias or whitewash. "I do have the feeling that these cases should be investigated more diligently and promptly. When misconduct investigations drag on, the public thinks there are more and more of them", says Edward Rall, director of intramural research.

Rall believes that much of the problem lies in the initial investigation by the university. "I'm not sure that all the universities understand that they can't cover up. When they close ranks, they only postpone the moment of truth."

Although NIH plans at present to retain the policy of letting the university do the first inquiry, it is fine-tuning other OSI procedures. Under consideration are 60-or 120-day deadlines — similar to the guidelines for university investigation — on the various stages of the OSI process. But it may be too soon to look for clockwork precision. Says Hadley, "the length of the investigations is one concern — but a lack of balance and objectivity is another."

G. Christopher Anderson

History of Australian prehistory

SIR—A recent News item by Tania Ewing (*Nature* **345**, 9; 1990) contains a number of inaccuracies and misconceptions about the department of prehistory in this school, apparently based on allegations made by Dr Johan Kamminga. One is that there has been a 'Cambridge cabal' ensuring that Cambridge graduates dominate the staff appointments and that British graduates get the more senior posts. The facts are that since its establishment in 1961 there have been only seven tenured appointments in the department of prehistory. When the first two tenured appointments were made in the early 1960s, there were no PhD graduates in prehistory from any Australian university. Any appointees would have required overseas qualifications. At the time of their appointments, tenured staff held a total of 16 degrees from courses of university studies. Of the 16 degrees, only four were from Cambridge. Five were from Sydney. In 1989, the nine academic staff (tenured and non-tenured) held a total of 21 degrees, three of which were from Cambridge. Five were from Sydney.

Neither is Cambridge dominant as a source of students. Of the 32 students who have completed PhDs in the department, only three took their first degree at Cambridge. Four were from Sydney from a total of 12 from Australian universities.

The assertion that Cambridge dominates is not supported by the facts. One could claim a Sydney network with more justification, but such a claim would be equally untrue. The school, including the department of prehistory, seeks to make the best possible appointment from applicants on the basis of merit, not place of origin.

A major misconception in the article leads to the implication that, following the 1978 review of the school, the department of prehistory is at fault in not shifting its interest towards South-East and East Asia. This was not a general requirement throughout the school. Neither was recruitment of students from these areas a requirement, and indeed has never been a deliberate policy. The school takes students, and awards scholarships, on a competitive basis from all over the world, without any regional preferences. The department of prehistory recruits students interested in working in its field areas, Australia and Melanesia, regardless of the place of origin of the students.

The assertions that electoral committees have been "stacked", that Australian graduates get junior posts while "British graduates get the more senior positions" or that the department of prehistory in this school is a "Cambridge academic colony" are quite simply untrue.

R. GERARD WARD

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SIR—Before your readers conclude that the prehistory department at the Australian National University is a 'Cambridge circus' and especially before there is a rush of Cambridge graduates looking for certain tenured employment in Canberra, I hope you will allow an all-Australian member of the department to correct Tania Ewing's report.

No member of the department has seen Dr Johan Kamminga's allegations so it is difficult to judge whether they are all as inaccurate as the ones he makes public.

In its 21-year history, this research department has had only seven tenured academic staff. Four had Cambridge undergraduate training but two of these obtained Australian PhDs as the basis of their appointment here.

Jack Golson and John Mulvaney were recruited from Auckland and Melbourne respectively to found Australia's first prehistory department in 1969, so by definition their training was non-Australian. It is not surprisingly that they both received it at Cambridge, then, as now, one of the most distinguished centres of teaching and research in prehistory.

Golson and Mulvaney's ethical and research standards have been major reasons behind the dramatic expansion of prehistory in this region and why the indigenous people of the area have become so interested in it.

It is therefore outrageous for Kamminga to claim that these scholars, two of the world's most respected prehistorians, 'fiddled appointments' from the time they arrived in Canberra.

The next tenured post in the department was not filled until 1972, but your readers can make their own assessments as to whether the subsequent tenured staff — Rhys Jones, Jim Allen, Doug Yen, Matthew Spriggs and myself — had to be 'fiddled' or were appointed because of our publications and research skills.

It is interesting that Kamminga's degrees, like my own, are from the department of anthropology in the University of Sydney. Of the seven tenured prehistorians at our *alma mater* over the past 29 years, all but two have had at least one Cambridge degree. I am certain though that, like us, they simply make the best appointments on the increasingly rare occasions when there is a vacancy to fill.

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Ungodly thoughts

SIR—Am I alone in being surprised at the number of debates, in the pages of *Nature*, on religion and the existence of god? Most recently this was apparent in the review of Sir John Eccles' new book (*Evolution of the Brain: Creation of the Self*: *Nature* **344**, 117; 1990), and the subsequent reply from god (as revealed to Ralph Estling, **344**, 582; 1990). Although, as in this example, references to god in *Nature* are usually frivolous, this is not always the case (see, for example, **337**, 498; 1989), and the ways in which religion affects the world at large are certainly not a laughing matter (as I am sure Mr Salman Rushdie would testify). Yet *Nature* seems to be according respectability to religious beliefs.

As scientists, should we not be protesting at the way religions permeate society and intrude into our lives, whether or not we want them to? Or, if not protesting, at least making our views known? In a straw poll that I conducted in this medical school, only eight of the 63 people I questioned had any form of religious belief. I suspect that this low incidence of religious beliefs is true of scientists in general, but it would be nice to know for sure.

I would like to suggest that *Nature* should conduct an opinion poll on the religious beliefs of its readers. If my small sample was typical, such a poll could indicate the strength of the atheist lobby (the silent majority?) in science. What would this achieve? Probably nothing, but who knows? Governments might resolve to bring to a halt the religious indoctrination of children. If children were informed on how many different religions there are, and on the fact that if any one of them happened to be right, all the others would be wrong, they might without much difficulty deduce the liklihood that all religions are wrong. Religious programmes on radio and television could come with a government health warning. People might even begin to realize that what happens to our world depends on us.

Nature would be performing a valuable public service by such a survey. The international readership of the journal would enable us to ascertain whether the prevailing religion of different countries influenced the extent of rejection of religious belief by scientists. Whatever the outcome, it would be good to know how many of us think that man created god (in his own image, of course).

CHRISTOPHER J. LOTE

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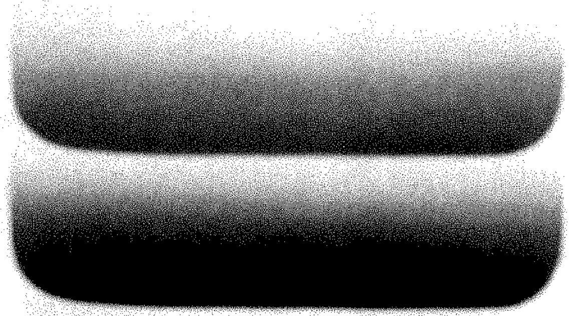

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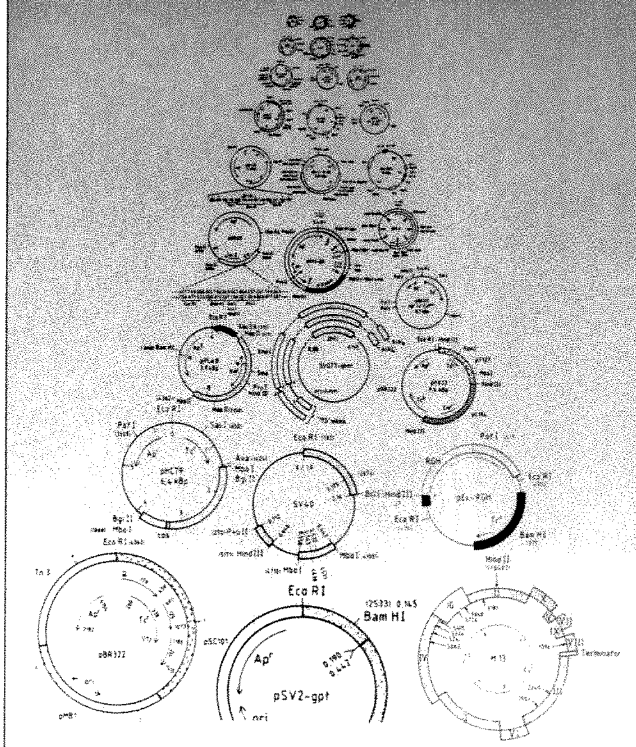

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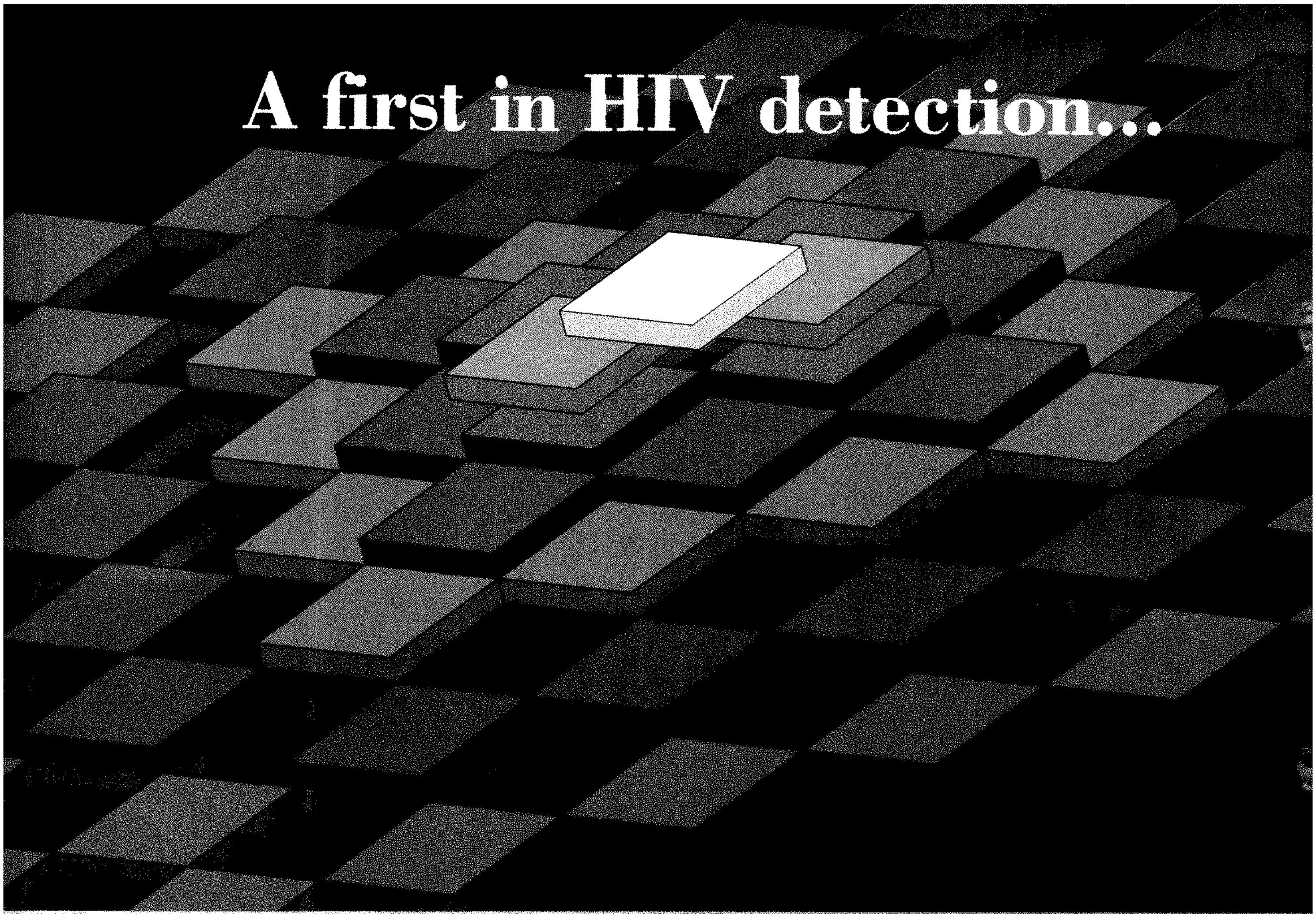

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Carbon dioxide will be harder

Last week's successful review conference of the Montreal Protocol on ozone would have served an even better purpose by spending more time on verification and compliance.

LONDON had hardly said goodbye to last week's gathering of the parties to the Montreal Protocol on ozone than it was filled with the delegations of the members of the North Atlantic Treaty Organizations, who will be discussing (among other things) the prospects for further arms control measures in Europe. There seems little doubt that the people at the earlier gathering are a more trusting lot than those at the second. What does either group have to learn from the other?

For decades, it has been taken for granted that there can be no arms control without verification. The assumption has always been that the stakes are too high to take the other fellow's word on trust. This is one of the reasons (there were several others) why, in 1979, the then US President Jimmy Carter did not send the SALT II treaty (on strategic arms) to the US Senate for ratification: as negotiated, it allowed only for 'national means' of verification, which was a euphemism for the use of surveillance satellites. By contrast, the treaty on nuclear missiles of intermediate range, signed at the end of 1988, and the START treaty now being negotiated, include provisions for the direct inspection of disarmed missiles, sometimes on unprovoked demand.

The Montreal Protocol does not yet have teeth like that. Even in its newly tightened form (see page 6), the convention is not especially specific about the the baseline data, for 1986 and 1989, against which future reductions of chlorofluorocarbons (CFCs), and variants on that theme, are to be assessed. The secretariat requires of the governments that are members of the convention that they supply details of production and consumption of the various materials interacting with ozone in the stratosphere. And where will those data come from? The only possible sources are the companies that manufacture them, and which must be supposed to have a vested interest in inflating their first estimates so as, later, to be less hurt by the proportionate reductions to which they have agreed.

At least on past form, the arms controllers would not so easily have taken what their fellow signatories had to say about their own dispositions as synonymous with the truth. In the absence of 'national means of verification', there would have been calls for lists of the manufacturing plants at which offending operations were being carried on, as well as for opportunities for inspecting them. For what it is worth, the signatories of the Montreal Protocol might reasonably have bound themselves to provide a list of the plants manufacturing CFCs and the other substances now controlled, if only so as to be better able to tell what to make of newly constructed plants if they should make their appearance in the years ahead.

It is also surprising that the Montreal Protocol is as innocent as it seems of formal arrangements for the systematic monitoring of the now-controlled materials. The ostensible objective of the enterprise is to regulate the concentration of these chemicals in the stratosphere, for which purpose the tropospheric concentration is a legitimate proxy. But the measurements by which the supposed increasing concentration of, for example, CFCs has been verified have mostly been carried out with air samples collected from oceanic islands.

That makes sense if the objective is to detect trends unencumbered by locally generated noise, the nearness of a chemical plant for example. Now that the convention has been made more stringent and its scope extended, its members cannot be expected to continue to rely on whatever measurements of this kind independent investigators choose to carry out, but it is plainly of more than passing interest to them to know something about sources of regional or even local noise that may betoken the presence of unsuspected chemical factories.

These issues have been given close attention over the years in negotiations to control the manufacture of chemical weapons, mostly at Geneva (in the building in which the United Nations Environmental Programme has its European office). Not so long ago, the Finnish authorities explored the use of civil aircraft equipped with air samplers and gas chromatography as a means of telling what kinds of chemical plants lie beneath civil airline routes. Fitting the exhaust stacks of all chemical factories with sealed automatic samplers would be more effective, but would encounter the objection raised, during the test-ban negotiations in 1978, that remotely operated seismometers would provide much more extensive data than were required for the working of the treaty. Even in the cause of the Montreal Protocol, not every chemical manufacturer would welcome others knowing what flows up his chimney stacks.

Yet another lacuna in the new arrangements is financial, and concerns the procedure for recompensing developing countries for their willingness not to make CFCs, but only the substitutes for them, and to lag no more than ten years behind the rest in full compliance. It is, of course, remarkable that so many rich countries have agreed to contribute to what is called the multilateral fund to compensate developing countries for their self-denial. But it will be a headache for the lawyers and the accountants. The preamble to last week's conference document records the need to avoid "double-counting". But only trusting folk like the ozone people would have let things get this far without calling in teams of lawyers to draft their resolutions.

That is as it should be. It would have been absurd to have invested the ozone negotiation with all the niceties of the arguments there have been in the avoidance of nuclear war. That does not imply that the ozone problem is not serious, but that it is a less serious problem than the threat of nuclear war. And, as the signatories of the Montreal Protocol openly confess, the last thing on their agenda was to frighten off non-signatories by too elaborate a system of control. Fair play, the strategy has worked. It is a victory that China and India may now join.

So why rehearse the gaps in the convention that will send the world's arms controllers into shivers? Because the convention is more than a means of reducing the incidence of skin cancer, it is a model for the convention there will soon have to be on the control of the emission of greenhouse gases (among which the CFCs are incidentally important in their own right). And there the stakes will be much higher, perhaps even comparable with the threat of nuclear war. People will be less willing than over ozone to trust others to tell the truth, from the fear that they may be lying.

That is why a few arms controllers might usefully get together, in the wake of last week's successful conference, to advise the ozone people on the ways in which their protocol might have been made not just a declaration of good intentions, but watertight as well. The exercise would of course be unnecessary and thus academic, but a version of it will be needed when work begins on carbon dioxide.

John Maddox

PULSAR ASTROPHYSICS

Good things come in threes

Charles Bailyn

THE high density of globular star clusters is believed to be conducive to pulsar formation. But although recent years have indeed seen a stream of pulsar discoveries in these systems (19 are now known, whereas none were three years ago), the observations continue to bring forward as many questions as they answer. The discovery by Anderson *et al.* of the second and third pulsars in the very dense cluster M15 (Fig. 1), reported on page 42 of this issue[1], is especially significant, both for the innovative method used to detect them and for the interesting properties of the two pulsars and of the cluster itself.

Pulsars are rapidly magnetized neutron stars. They emit beams of electromagnetic radiation from the magnetic poles, which are observed on Earth as pulses of radio emission as the beam sweeps across once with each rotation. The pulsars in globular clusters belong to a subclass often referred to as 'millisecond' pulsars, although in fact their observed pulse periods range from a few milliseconds to a few tenths of a second. The search for pulsars usually involves taking a Fourier transform of a long time series of radio measurements to look for a periodic signal amidst the background. This search must be made for different values of the dispersion measure — the amount of ionized gas between the observer and the pulsar — because the speed of light for a given frequency depends on the column density of this intervening plasma. Without a correction for this effect, the pulse arrival time will vary with frequency, resulting in an undetectable 'dispersed' pulse. But there is little, if any, gas within globular clusters, so the dispersion measure is the same for all pulsars in a given cluster. Anderson *et al.* have taken advantage of the previous discovery[2] of a pulsar in M15, from which was determined the dispersion measure towards the cluster, to greatly increase the sensitivity of their search, which has resulted in the discovery of two fainter pulsars.

The improved sensitivity comes about in two ways. First, long series of data can be analysed with much less computing effort, as only one value of the dispersion measure need be considered. Second, a strong 'selection effect' that normally mitigates against finding pulsars in binary star systems with short orbital periods can be at least partially removed. The selection effect arises because the observed pulse period varies, and the signal in the Fourier transform is consequently reduced, as the pulsar in a binary system is accelerated relative to the Earth by its orbital motion. Anderson *et al.* have used the improved computational efficiency made possible by knowing the dispersion measure to search for the variable pulse periods that would be created by a range of constant accelerations. This procedure resulted in the discovery of the third pulsar, PSR2127+11C, which could not otherwise have been detected.

Indeed, it is the short orbital period (about eight hours) of PSR2127+11C that makes its discovery particularly exciting. Its orbital parameters are remarkably similar to those of PSR1913+16, which has been used for a sensitive test of general relativity[3]. The exquisite accuracy possible for timing measurements on short-period pulsars means that it is feasible to look for various relativistic effects, including the decrease in period of the orbit through emission of gravitational radiation. These effects should be observable in PSR2127+11C within a few years.

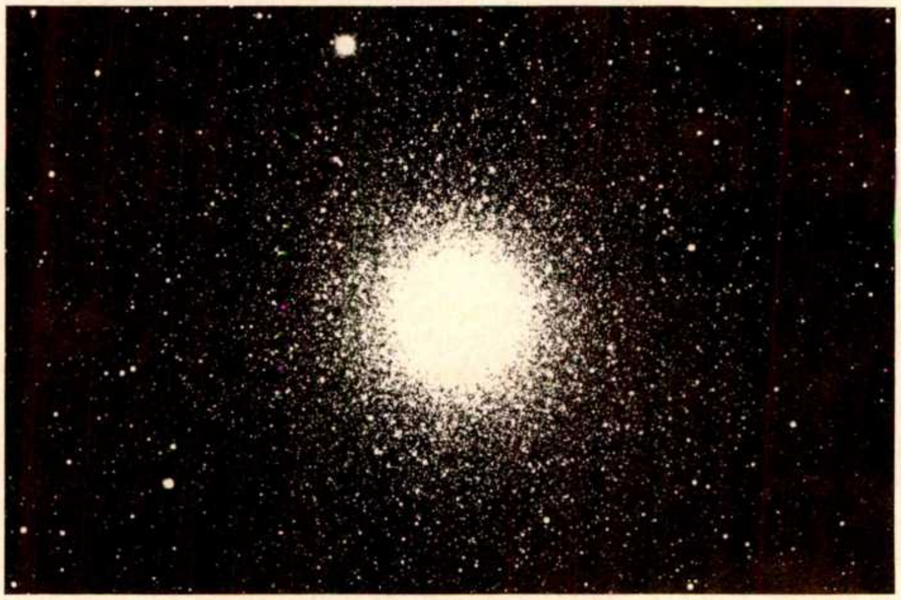

FIG. 1 An optical image of M15 taken at the Kitt Peak National Observatory. The two pulsars (A and B) for which accurate positions are known are located at the centre of the bright region.

M15 is itself an interesting cluster, being the best-studied member of a class of clusters that have strong cusps in their central density (Fig. 2). These cusps are a signature of an advanced stage of cluster evolution. The high central density should give rise to a large number of stellar collisions and near misses, which are thought to result in the formation of accreting neutron stars in binary systems — the precursors, according to the standard scenario, of millisecond pulsars. Thus M15 should be a fertile breeding ground for pulsars, as the new observations seem to confirm.

The three pulsars in M15 provide probes of the cluster's dynamics. The first of them[2] shows an unprecedented increase in spin rate with time — all other pulsars are slowing down. This is not thought to be an intrinsic property of the pulsar, but instead a result of the strong gravitational acceleration caused by other stars in the cluster. Thus measurements of pulsar timing can probe the cluster's gravitational potential. The locations of the pulsars within the cluster reveal the operation of dynamical processes: pulsars A and B, for which positional information is available, are within a few arcseconds of the cluster centre, indicating that these relatively massive systems have fallen there as equipartition of energy has become established through interactions with other cluster stars. Finally, the fact that two of the three pulsars in M15 are not binaries is in itself revealing in terms of their dynamical interactions with other stars. Although millisecond pulsars are thought to be born in binary systems, a recent study[4] of the dynamics of M15 shows that encounters with a third star are likely to lead to the 'ionization' of a binary pulsar (that is, the loss of its companion).

But the stories told by pulsars about their parent clusters are not limited to the clusters' dynamics — they also reveal problems with generally accepted theories of stellar evolution. The basic difficulty is that there are so many globular-cluster pulsars. As distances to globular clusters are large, only the very brightest pulsars will be observable; thus the pulsars observed probably represent only a small fraction of the total population. Recent extrapolations[5,6] suggest that there may be as many as ten thousand pulsars in galactic globular clusters. Neutron stars are created in supernova explosions of stars with ten or more times the mass of the Sun, so this large number of pulsars requires a large primordial cluster population of massive stars. In M15 and several other clusters, however, studies[7] of the remaining unevolved stars (all less massive than the Sun) show that the number of stars drops off very sharply with increasing mass. Thus the function that describes the variation with mass of the frequency of occurrence of stars must be more complicated than the simple power law that is usually assumed.

This requirement for a large number of neutron stars also poses problems for the stability of the clusters as a whole, because the mass loss during the evolution of a large population of massive stars has been shown to result in complete disruption of the cluster[5]. Furthermore, neutron stars in binaries are spun up to 'millisecond' periods by accreting material with high angular momentum from their companions. Such accreting neutron stars are indeed observed in globular clusters as strong X-ray sources, but estimates of the birthrates of these systems are orders of magnitude too small to account for the inferred number of pulsars[5,6].

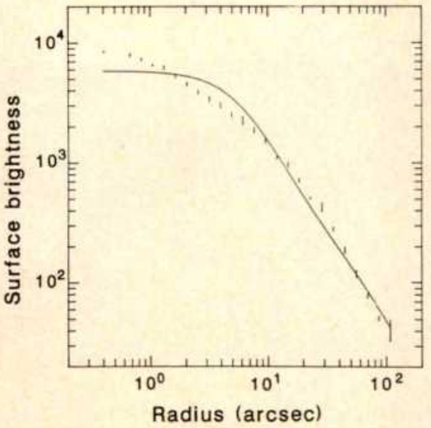

FIG. 2 Radial density profile of M15 (from ref. 9). At small radii, the data (short horizontal lines) considerably exceed the predictions of the King model[10] (solid line), which fits most globular clusters.

Now that we know where and how to look for millisecond pulsars, we can expect the stream of discoveries to become a torrent. There should soon be many more opportunities to exploit the unique tests of gravitational theory provided by pulsars, as well as to learn more about the evolution of stars and star clusters. ☐

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QUANTUM MECHANICS

No need for nonlinearity?

Richard Thompson

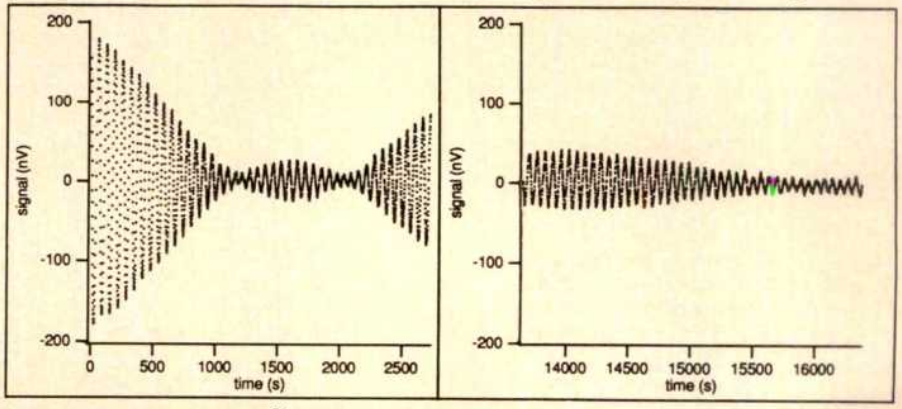
CLASSICAL oscillatory motion, such as that of a simple pendulum, can become nonlinear at large amplitudes, whereby a dependence on amplitude starts to creep into the resonant frequency. Last year Weinberg[1] proposed that similar nonlinear corrections might be necessary to the wave equations of quantum mechanics: this idea was tested subsequently to high accuracy in a study of trapped beryllium ions by Bollinger *et al.*[2]. Chupp and Hoare[3] have now reported a similar test from a different kind of experiment based on spectroscopy of the ^{21}Ne nucleus. They find that any nonlinear corrections to the usual linear quantum-mechanical formulation must be tiny: smaller than 1.6×10^{-26} of the binding energy per nucleon in ^{21}Ne atoms.

Although quantum mechanics has no difficulty in providing a description of nonlinear effects, Weinberg[1-4] revived the question of whether the usual linear form of quantum mechanics is complete. He proposed a class of possible corrections to the usual hamiltonian function, which is linear in the product of ψ and ψ^* (the wavefunction and its complex conjugate). These corrections are nonlinear in the product of ψ and ψ^* and can be cast in such a form that the hamiltonian still describes separated systems correctly. The consequences of such corrections can be calculated and tested in the laboratory; but there is as yet no experimental evidence that they are necessary. One particular consequence would be that the principle of linear superposition of wavefunctions would no longer hold. It is this aspect that the experiments set out to test.

I have discussed the Be$^+$ experiment previously[5]. It involved the investigations of an essentially two-level system within the hyperfine structure of the ground state of ^{9}Be$^+$. A cloud of ions was held in an ion trap and cooled by collisions with laser-cooled Mg$^+$ ions held in the same trap. The ions were prepared in a superposition of the two levels by a microwave pulse and the precession frequency of the nuclear spin in an external magnetic field was measured to very high precision. Nonlinear corrections to quantum mechanics would show up as a dependence of the precession frequency on the degree of excitation of the ions — the frequency should change as the amplitude of the excited state in the superposition state is changed. No nonlinearities were detected at the level of 4 parts in 10^{27} of the binding energy per nucleon of the ^{9}Be nucleus.

Chupp and Hoare[3] have chosen to study the ground state of the ^{21}Ne atom, which has a nuclear spin of 3/2. The atoms are placed in a weak (about 3 gauss), uniform magnetic field B, which splits the ground state into four levels (labelled by the quantum number M_I) such that the three transition frequencies between pairs of adjacent levels are all about 995 Hz. These three frequencies are monitored as a function of the degree of alignment ('tensor polarization') of the system: a measure of the degree to which the system is in the outer ($M_I = \pm 3/2$) states rather than the inner ($M_I = \pm 1/2$) states. Nonlinearities of the simplest form to satisfy the conditions mentioned earlier will cause these frequencies to vary in a particular manner.

Assuming that the nonlinearity is characterized by a small parameter ε (with dimensions of energy), each of the four M_I levels is shifted by an amount proportional to ε and dependent on the exact state that the system is in. If ω_1 is the transition frequency between the $M_I = 3/2$ and 1/2 levels, and ω_3 is the transition frequency between the $M_I = -3/2$ and $-1/2$ levels, it turns out that the difference frequency $\omega_1 - \omega_3$ is equal to $(2+T_2)\varepsilon/\hbar$, where T_2 is the alignment of



Coherent free precession of ^{21}Ne over 4.5 h. The signals show beats due to the tiny differences between the three transition frequencies for the four-level system. The traces can be fitted to theoretical curves to extract the three frequencies and their damping coefficients; the degree of alignment can be extracted from the relative amplitudes of the frequencies.

the system. Thus if the system is prepared in superposition states with varying amounts of alignment, this difference frequency should change if nonlinearities are present. The technique, developed from earlier experiments that searched for violations of Local Lorentz Invariance[6], uses a cell containing both ^{21}Ne and ^{3}He. The nuclear spin of ^{3}He precesses in the magnetic field, but shows no nonlinear effects as it has a spin of only 1/2. The precession frequency is about 9,600 Hz, which is divided electronically by 9.649 to give a reference frequency about 1/60 Hz away from the ^{21}Ne precession frequency. The beat signal between the two frequencies can be followed for a period of 2,700 s before the He signal decays. The Ne signal can, however, be measured over a period of 4.5 h if the helium atoms are re-excited periodically. The neon atoms are initially polarized (put into specific spin states) via spin-exchange collisions with Rb atoms excited by laser irradiation. Once the polarization is established, the cell is cooled to freeze out the Rb vapour, and a pulse of resonant oscillating magnetic field tips the neon spins by 20°. Their free precession is then monitored with a pickup coil. A critical feature of the experiment is that any small variations in B do not influence the results, as the helium atoms providing the reference frequency sample the same field as the neon atoms. The important difference frequency is then relatively insensitive to the field fluctuations.

A typical trace of the beat signal is shown in the figure. From these traces, $\omega_1 - \omega_3$ can be found as a function of T_2, giving a limit for ε/h of 12 ± 19 μHz. Comparing this with the binding energy per nucleon of the ^{21}Ne nucleus gives a fractional nonlinearity of less than 1.6×10^{-26}, similar to the Be ion-trapping experiment.

What does all this signify? The Weinberg model has been designed to incorporate nonlinearities in as general a manner as possible, but it is of course possible that a different model is needed. It is also possible that nonlinearities are present but only at an even smaller level; in that case their detection may not be feasible, as these experiments are already extremely sensitive because of the very long interaction times involved. But it seems most likely that a linear quantum-mechanical description of matter is sufficient and that nonlinear corrections are not necessary. □

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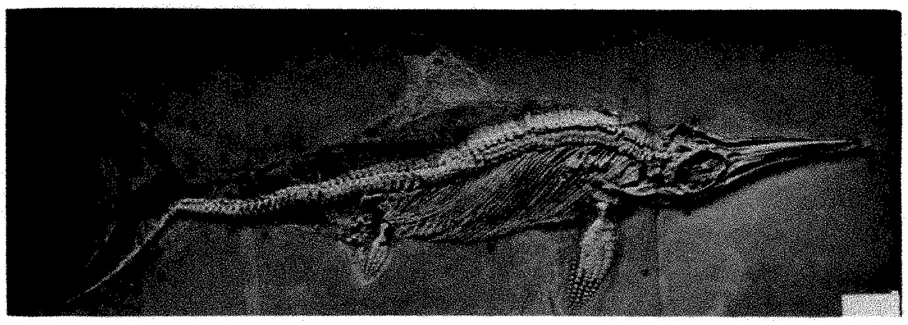
PALAEONTOLOGY

Two dietary developments

Michael A. Taylor

Kipling[1] traced the origin of the baleen whales to one which unwisely ate a "shipwrecked Mariner of infinite-resource-and-sagacity", who cut up his raft into a small square grating and rammed it down the animal's throat. Today's hypotheses for the origin of major vertebrate groups are tested rather more rigorously by fossil evidence and internal logic. Two recent and apparently disparate examples show how illuminating that approach can be where a group's key adaptation has changed its function, or when the original shape of members of a group has been transformed. In one report[2], Bodmer argues that the stomach of ruminant mammals such as cattle and sheep, now used to digest grass and fibrous plant material, evolved to deal with tough fruits. In the other[3], Massare and Callaway show that ichthyosaurs, the marine reptiles famous for resembling sharks and dolphins even down to the deep double-lobed tailfin, did not even have tail fins to start with, but that such tails became advantageous with a change in feeding patterns.


University Museum, Oxford

Ichthyosaurs — these creatures may have evolved from shallow-water predators.

The stomach of ruminants contains a compartment, the rumen, in which plant food is fermented by symbiotic microorganisms in between regurgitation and further chewing (as the cud). Otherwise indigestible fibre can thus be broken down into assimilable nutrients: this has enabled ruminants to become today's dominant large herbivores feeding on fibrous grass or browse. But fermentation tends to waste free nutrients already present in high-quality food such as fruits, so that the ruminant stomach would seem to be a handicap to frugivores. Fossil evidence indicating that the first ruminants fed on fruits in Eocene tropical forests (50–42 million years ago) seems, therefore, strange.

Today's closest equivalents to the original ruminants are the red and grey brocket deer (*Mazama americana* and *M. gouazoubira* respectively), small Amazonian forest frugivores. They feed especially on palm fruit, the seeds of which are highly nutritious but heavily protected against attack. Most mammals that eat them break or gnaw through the shell, but the deer simply swallow the seeds whole for digestion and softening by rumen microorganisms, before rechewing.

In Bodmer's view[2], the ancestral ruminant stomach originally evolved to cope with such tough fruits, and then became used increasingly to digest other fibrous plant parts such as leaves. Interestingly, brocket deer show this dual use, eating more nonfruit material when stranded on temporary islands during floods. As the Eocene forests were very similar to Amazonia today, this is a tempting model for the transition.

The diversification of ruminants during the Tertiary coincides with the decline of nonruminant ungulates, not because they were inherently superior, but probably because climatic and vegetational changes, such as the development of grasslands, favoured their ability to process small amounts of food efficiently (the nonruminants' form of intestinal fermentation requires more food and a greater throughput of it)[4]. But nonruminants can be superior under different conditions. So the new hypothesis[2] has the merit of invoking a specific qualitative advantage of rumen fermentation: the fruits can be fermented and then returned to the mouth for further chewing in a manner which would be impossible for otherwise efficient hindgut fermenters, unless they consume their own faeces: hardly practical for a large, mobile mammal.

In the second example, Massare and Callaway[3] use new data on ichthyosaurs from the Triassic period (248–213 million years ago) to conclude that ichthyosaurs are most probably members of the Diapsida, a large group of reptiles characterized by the presence (in most) of two pairs of openings in the sides of the skull behind the eyes, for jaw muscle attachments. If so, ichthyosaurs must have lost the lower pair of openings. Their origins lie among the primitive lepidosauromorphs (the diapsid group including tuataras, lizards and snakes), and the apparent discrepancies — such as the lack of a breastbone, present in primitive lepidosauromorphs

— seem to be related to their transformation into aquatic reptiles.

A near-monopoly of marine reptiles in the Mesozoic (250–65 million years ago) was thus enjoyed by the Diapsida, which apparently include the placodonts[5] and nothosaurs (and by implication plesiosaurs)[6,7], as well as the sea-crocodiles and mosasaurs (giant marine lizards). Was this just luck, or was the diapsid skull particularly effective for aquatic feeding?

Paradoxically, answers to this question are best answered by examination of the postcranial skeleton. Primitive diapsids retained the basic reptilian terrestrial locomotor pattern of side-by-side undulation of the body and tail, increasing the thrust of the limbs, while the powerful tail muscles helped to pull the hindlimb backwards. This basic undulation is also used for swimming, with the limbs trailing. Triassic ichthyosaurs had longer and more flexible necks and bodies than the more dolphin-shaped forms that evolved later. The Triassic forms must have swum by the same undulation of the body and tail that propelled their terrestrial ancestors. A relatively broad, long tail fin, which was an enlargement of the natural keels of the tail, helped generate thrust over most of the animals' length. In contrast, later forms relied mainly on tail movement with force produced by rapid oscillation of an enlarged, bilobate or even crescent-shaped tail fin. This was ideal for maximizing thrust and minimizing drag in steady swimming — perfect for cruising or fast pursuit of prey in open water.

But Triassic ichthyosaurs were not inferior intermediates between terrestrial reptiles and Jurassic ichthyosaurs. The long body and tail permitted large amplitude oscillations and rapid acceleration — useful for ambush predation — and the broad tail gave a burst of power for rapid starts[3]. Massare and Callaway thus envisage ichthyosaurs as evolving from shallow-water, elongate, crocodile-like predators — in other words, little more than a standard reptile — through a more aquatic but still elongate coastal ambush predator, to the dolphin-like open-water, deep-bodied predators of the open sea. The high degree of adaptation of the intermediates is a particularly pleasing refinement to what was thought to be a well-understood evolutionary development.

The new reports show what insights can be garnered from a marriage of palaeontology and the comparative anatomy of function in living animals. They are, of course, just two of many examples, which together constitute a scientific success story of which Kipling would surely have approved. ☐

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EVOLUTION

Mountaineering with microbes

Daniel E. Dykhuizen

MICROORGANISMS are well suited to experimental studies of evolution. They have such short generation times that observations of evolutionary change over thousands of generations are possible not only within the lifetime of a scientist, but even within the duration of a grant. Such experiments often give rise to surprising and unexpected results, as demonstrated by the report of Bennett, Dao and Lenski on page 79 of this issue[1].

The authors adapted a population of *Escherichia coli* to growth at 37 °C in a minimal medium by serially transferring the population for 2,000 generations. During this time, the population became well adapted to its environment. From this population a single cell was isolated. The clone derived from the cell was split into six experimental and six control populations, the control populations then being grown in the same environment as before — minimal medium at 37 °C. The experimental populations were grown in the same way and in the same medium, but at 42 °C. After 200 generations, the experimental populations had evolved. In the control populations, there was no measurable change, even after 400 generations. So far there is nothing unexpected in this: rapid evolution in a new environment; none in an environment to which the cells are already well adapted. What might not have been expected is that such similar environments — only 5 °C difference in temperature — lead to different evolutionary responses. The surprise is that the populations evolved at 42 °C were also fitter at 37 °C than the control strains.

Evolutionary biologists have a mental picture of the adaptive process. Fitness is represented by smooth-surfaced mountains, and an adaptive mutation allows a species to climb a little higher on the mountain. If the environment does not change, the species will approach the peak, and as it does so we expect the advantageous changes to become rarer and smaller in effect. In the experiment carried out by Bennett *et al.* this occurred during the first 2,000 generations at 37 °C such that there is no evidence for any further adaptation after 1,500 generations. So it seems safe to assume that the population is sitting near the top of the adaptive peak.

If the environment is changed, the topography will be remoulded. New peaks will arise from the slopes of old mountains and in place of old peaks there will be valleys. In this case, we could imagine a new, higher peak arising out of the side of the old peak. The population now evolves towards this peak. When this population is returned to the former environment, it would be expected to be less fit, rather than more fit.

The authors offer two hypotheses to explain their results. First, that the mutations selected at 42 °C are not present at 37 °C in sufficient frequency to be selected. This is possible. But then the adaptive path is dependent upon the available mutations even in large populations where most single mutations would be expected within a few tens of generations. If this is generally the case, the metaphor of the adaptive landscape must be restricted to genetic variation currently in the population. Second, that because of the smaller selection coefficient, the mutations did not rise to a substantial frequency at 37 °C. Again this is possible, but the average fitness increase at 37 °C was 4 per cent, half that at 42 °C. Thus, if these mutations dominated after 200 generations in the 42 °C populations, they should have appeared in the 37 °C populations after 400 generations. Where were they?

I offer two more hypotheses. One is that various epistatic mechanisms are involved. For example, assume that two mutations are required for the increase in fitness at 37 °C. Neither of these mutations alone is advantageous at this temperature, but at 42 °C at least one is advantageous by itself and in combination more advantageous. This proposal also has flaws. It would take between 100 and 200 generations for the first mutation to reach frequencies where the second mutation would happen in the same cell. Then there should be a fitness increase in the period from generation 200 to 400, which was not seen. My other hypothesis is that the selection is frequency dependent. When the mutations are rare, they have very little advantage at 37 °C, but considerable advantage at 42 °C. Further explanations can be added to explain the authors' result, but when one starts piling up hypotheses in this way it is hard to stop. Here we have a warning signal that reality does not agree with our picture and investigation of mechanism becomes a necessity.

This is not the only case of unexpected results in experimental studies of evolution. Paquin and Adams[2] found that a population could evolve such that it became less fit than the starting strain.

Saccharomyces cerevisiae were cultured in a chemostat, a device in which microorganisms can be continuously grown in a nearly constant environment[3]. Every time an adaptive mutation swept through the population, strains carrying this adaptation were isolated and competed against ancestral strains, either the one used to start the population or ones carrying previous mutations. Each strain was fitter than the previous one — the strain carrying the first adaptive mutation was fitter than the starting strain, and the strain carrying the second adaptive mutation (and probably also the first) was fitter than the one carrying only the first. But the strain carrying the third adaptive mutation was selected against when set in competition against the starting strain. So selection is not transitive as the mountain model of fitness assumes it to be. To continue the mountain analogy, this result shows that, as the population climbs a mountain, it can change the topography radically even though the physical environment remains constant.

Even in the simplest system — *E. coli* growing in glucose-limited chemostats — unexpected results occur. Chemostats are designed so that a single nutrient limits growth and the organisms compete for this nutrient. Helling *et al.*[4] used the system to study the adaptive evolution of *E. coli*, expecting to isolate a series of mutations which made the cells increasingly fitter in this constant environment. The approach should have allowed definition of the path to the peak; instead, the original strain 'speciated' into two separate lineages, both being maintained on what seemed to be a single limiting resource. Closer investigation showed that they had evolved such that the resource was divided — one lineage used the glucose, and excreted compounds on which the other lineage could grow. So the image of a rock-hard mountain decomposes into many fitness landscapes, which in turn continually split into new ones as organisms evolve. ☐

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PHYSICAL CHEMISTRY

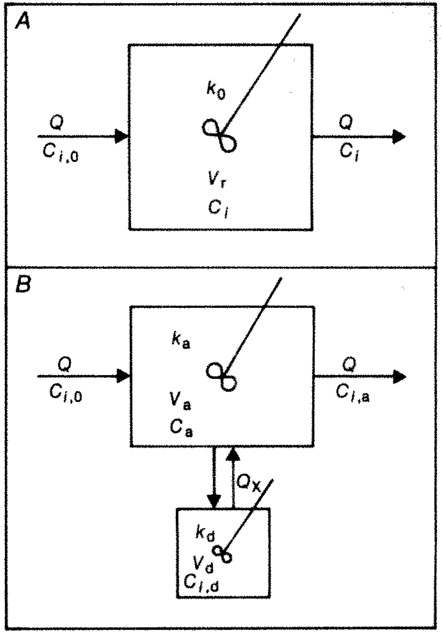
Shaken, stirred — but not mixed

Irving R. Epstein

THE glass stirring rod has largely been replaced by magnetic stirrers, shakers, ultrasonicators and other more sophisticated devices designed to produce homogeneous reaction mixtures in chemical systems. Most chemists carry out their reactions in the belief that any residual nonuniformities remaining in their reaction vessels have negligible effects on the results of their experiments. Evidence has been accumulating, however, that in at least some reactions with the kind of highly nonlinear kinetics that leads to several steady states or sustained oscillation, significant concentration gradients arise despite the experimenters' best efforts to eliminate them. Menzinger and Dutt[1] now report quantitative measurements of macroscopic concentration gradients in the oscillatory chlorite–iodide reaction carried out in a stirred continuous-flow reactor. Concentration differences of up to 800 per cent are found between points near to and far from the stirrer shaft.

Chemical engineers have appreciated for some time that such 'irrelevant' details as the placement of input jets, the shape of the stirrer or the orientation of baffles can influence markedly the output of certain reactor processes[2]. Roux *et al.*[3] showed the dramatic effect of imperfect mixing in nonlinear chemical dynamics when they found that, for the bistable chlorite–iodide reaction, the hysteresis limits (as a function of the rate at which reactants are pumped into the reactor) vary significantly as the stirring rate is varied between 550 and 700 r.p.m., a range in which most chemists would have been confident that the reactor was well mixed.

Several theories have been proposed to describe the interaction of mixing effects and chemical kinetics. Two ideas must be kept in mind. First, the effect of inhomogeneities will be most pronounced when the reaction is nonlinear. If the rate of the reaction is proportional to the concentration of reactant X, [X], and if this concentration is 1M in half of the reactor and 3M in the other half, the net (average) rate will be the same as if the entire system was at a concentration of 2M. If, on the other hand, the rate is proportional to $[X]^2$, the average rate will be proportional not to $2^2 = 4$, but to $\frac{1}{2}(1^2 + 3^2) = 5$: imperfect mixing increases the net rate by 25 per cent. Second, two kinds of mixing must be accounted for[4]: macromixing, in which parcels of fluid from different inlets must be combined to yield equal bulk compositions; and micromixing, in which molecular mixing occurs for inhomogeneities with sizes of the order of the smallest hydrodynamic turbulent length scale.



Models of a stirred-tank reactor. *A*, Ideal, assuming perfect mixing. *B*, Macromixing effects included by postulating an active zone (a) which communicates with the input and output reservoirs, and a dead zone (d) which communicates only with a. The C_is are concentrations of reagent *i*, *k* is the rate constant, *V* is the volume and *Q* is the flow rate.

In one family of models (see figure), which can account for much of the qualitative behaviour, a reactor is pictured as a combination of a well mixed or active (a) zone and a poorly mixed or dead (d) zone. In this model[5], macromixing (but not micromixing) is described by parameters that determine the rate of exchange between the two zones (Q_X) and their relative volumes (V_a/V_d). Better mixing corresponds to a smaller dead zone. Such a model has been employed in recent efforts to explain the origin of chemical chaos in the Belousov–Zhabotinsky (BZ) reaction. In a somewhat controversial interpretation, Györgyi and Field[6] suggested that the observed chaotic behaviour[7], although genuine, arises not from the homogeneous chemical kinetics but from the interaction of these kinetics with imperfect mixing. This view, which was inspired by the inability of chemically reasonable models of the reaction to yield

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truly chaotic behaviour, has been tempered by a recent calculation[8] which indicates that a plausible model of the BZ reaction can indeed generate chaos under homogeneous conditions. It now seems likely that chaos in chemical systems may have two sources — complex homogeneous kinetics and/or interactions between kinetics and mixing.

The work of Menzinger and Dutt[1] is unlikely to be the last word on the nature of mixing effects in nonlinear chemical kinetics. The 0.25-mm microelectrode that they use to monitor spatial and temporal variations in electrochemical potential gives only very crude spatial resolution; a recent study of pattern formation in the BZ reaction[9] uses electrodes with micrometre dimensions. By monitoring simultaneously two microelectrodes at different locations in the reactor, Menzinger and Dutt find that when the reactor switches from one steady state to another, both the microelectrodes undergo a change in potential at the same time, even though their absolute potentials are quite different. On the other hand, certain chemical systems in a closed reactor show evidence of a transition between stable states that nucleates locally and then propagates through the system[10]. But although there are clearly questions still to be resolved, chemists are growing increasingly aware of the danger of assuming that they ever conduct their reactions in media of uniform concentration. ☐

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IMMUNOLOGY

Tamarin compatibility

Shirley A. Ellis and Andrew J. McMichael

OVER the past few years it has emerged that the major histocompatibility complex (MHC) class I region of human chromosome 6 contains expressed genes in addition to the (so-called classical) A, B and C loci[1]. The classical class I genes encode highly polymorphic transmembrane glycoproteins, whose main role is to present peptides from processed foreign antigen to cytotoxic T lymphocytes. To date, three other distinct loci (E, F and G) have been identified[2] and restriction fragment length polymorphisms suggest that there will be others. The origin and function of these genes have, however, proved elusive. On page 60 of this issue[3], Watkins *et al.* provide insights into the possible function of the products of one of these loci, HLA G, from a surprising source — the cotton-top tamarin.

The authors cloned and sequenced all of the MHC class I genes from a sample of 79 unrelated members of this species, and demonstrate that only 11 molecules are expressed. When these were compared with each other, they showed far less variability in amino-acid sequence than is seen between alleles of the class I loci in either man or mouse; ten out of the 11 molecules are more closely related to human HLA G than to classical A, B and C molecules. We must assume that these 'G-like' molecules are functional in the cotton-top tamarin, because the animals can mount cytotoxic T-cell responses. But when they encounter pathogens not found in their natural environment (Epstein–Barr, Rous sarcoma, measles and parainfluenza viruses, for example), they prove to be particularly vulnerable, a phenomenon which may be related to their lack of class I polymorphism.

We cannot assume however that because the cotton-top tamarin MHC class I appears to function normally, albeit in a limited way, the human HLA G has a similar role. There are several differences to be considered. The two full-length human G (derived) amino-acid sequences that have been reported[4,5] are identical; together with isoelectric focusing studies[6,7] this shows that there is probably no polymorphism (at the amino-acid level) at the G locus. The only cells that have been demonstrated to express the HLA G product are extravillous cytotrophoblast from the placenta and some trophoblast-derived choriocarcinoma cell lines. Thus the HLA G molecule is found only on cells that do not express HLA class I A, B or C. Possibly the most striking feature of HLA G which really sets it apart from classical HLA class I is its truncated cytoplasmic tail — the shortened tail is due to a termination codon at the beginning of exon 6 (the first cytoplasmic domain) that results in a smaller expressed product of relative molecular mass (M_r) 39,000. Although it does appear to be anchored in the cell membrane, this missing portion may have implications for the function of the molecule. The 'HLA G-like' molecules seen on the tamarin cells have a normal cytoplasmic tail, and are the size of classical class I molecules, M_r 43,000.

The human placental trophoblast cells, which are located at the maternal–fetal interface, are thought to play a part in the survival of the semiallogeneic fetus. It was believed that these cells formed an MHC-negative 'barrier' against maternal immune surveillance[8], but recent work has shown that they express high levels of HLA G (refs 5, 7). The function of this molecule is not known — it could be nonimmunological, related to the invasive properties of this tissue, or to the effects of peptide hormones. It may be necessary to have a 'neutral' HLA class I molecule, having a passive but important role, for example, to resist attack by natural killer cells[9]. Selection pressure against polymorphism of an HLA molecule expressed at this extremely sensitive site seems likely, and implies a function fundamentally different from that of the other HLA class I antigens, where a high level of polymorphism plays a crucial part in their immunological activities.

These results raise questions regarding the original nature and role of the ancestral HLA G genes. If the HLA G-like gene products functioned as classical class I molecules in the primate ancestor, they would have needed to 'lose' their polymorphism in adapting to a role in the trophoblast. Alternatively, the ancestral G genes might never have functioned as classical class I molecules; in the tamarin ancestor, accidental deletion of the classical loci may have resulted in an increase in polymorphism at the G locus and a wider tissue distribution. As Watkins *et al.* point out, that these primates are born as stable, bone-marrow-chimaeric twins may be relevant. This might help to compensate for the negative effect of the polymorphism of class I molecules expressed on the trophoblast; females might be tolerant of other parental haplotypes and would therefore be less likely to reject a fetus that carried a foreign MHC class I type.

We need to know therefore if the trophoblast cells in the tamarin express the same class I products as other tissues, or if they show limited or quite different expression. It will also be worthwhile to look more closely at other species that show limited MHC class I polymorphism, perhaps for different reasons (for example the cheetah[10] and the Syrian hamster[11]), to see if there too evolutionary pressures have acted in a similar way. ☐

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ORGANIC CHEMISTRY

New routes to natural products

John Mann

AT one time, the synthesis of complex molecules was the noblest pursuit for an organic chemist. During the past ten years, however, many organic chemists have been lured by the fat research grants and contemporary glamour of bio-organic chemistry. So it is good to see two excellent examples of natural-product synthesis reported by Ley *et al.*[1-7]. The biological interest of one of the compounds (*myo*-inositol 1,4,5-triphosphate (IP$_3$)) is well known, but that of the other (the anti-parasitic agent avermectin B1a), is also considerable.

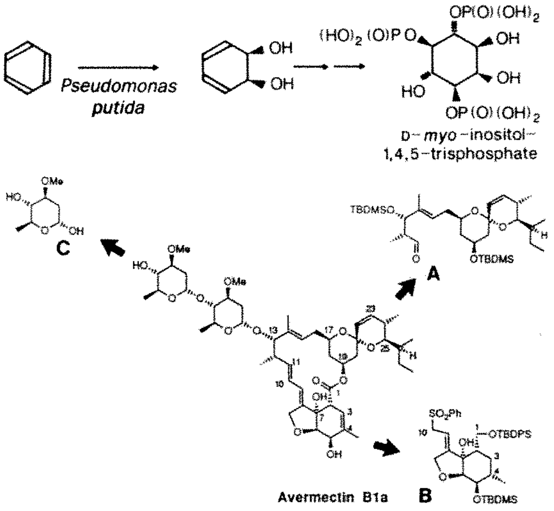
There has been enormous interest in the inositol phosphates, in particular the role of IP$_3$ as a second messenger, ever since their involvement in intracellular signalling was first proposed by Mitchell[8] in 1975. A number of syntheses of both the natural compounds and certain analogues have been described, but almost all of these commence with *myo*-inositol and proceed via a tortuous sequence of hydroxyl-group protections and deprotections in order to establish the desired phosphorylation pattern. Ley's approach[1-3] is conceptually different in that *cis*-3,5-cyclohexadiene-1,2-diol is used as the starting material (top in figure). This is produced from microbial oxidation of benzene by *Pseudomonas putida* and is thus cheap and readily available.

The remaining hydroxyl groups are introduced in a regio- and stereocontrolled manner to produce IP$_3$ and a large number of analogues. This new route is concise, does not use expensive or esoteric reagents, and provides access to the racemic forms of the compounds, or the pure enantiomers (both natural and unnatural) with only minor modifications. The approach makes a novel range of IP$_3$-related compounds readily accessible for biological investigation.

The avermectins are mould metabolites first isolated in 1976 from a strain of *Steptomyces avermitilis*[9]. They were shown to have high potency and broad spectrum of activity against animal parasites. The compounds seem to act at GABA receptors and to maintain the chloride channels in the open form, thus potentiating the inhibitory activity of GABA at neuronal junctions. Several avermectins are now produced routinely using fermentation technology, and ivermectin (the dihydro-analogue of avermectin B1a) is at present sold in more than 60 countries worldwide for the control of both internal and external parasites of livestock.

Notwithstanding this availability, the compounds are challenging targets for synthesis. Avermectin B1a, for example, has 20 stereocentres and a host of delicate functional groups; and to probe the biological activity of these interesting molecules, selected analogues must be prepared. Several research groups have accepted the challenge, but only one complete synthesis[10] of an avermectin has been reported previously.

Ley's new route[4-7] (bottom in figure) has the advantages of both conciseness and convergence of the various synthetic routes. The innovative chemistry devised to solve particular synthetic problems is the most interesting aspect of the work. Highlights include the use of organo-iron chemistry to produce not only the key spiroacetal (**A** in figure) but also the oleandrose subunits (**C**); and the coupling of the fragments **A** and **B** to form the aglycone. The final steps of the synthesis were achieved with the minimum of protection/deprotection of hydroxyl groups by an elegant exploitation of the selective reactivities of these groups. The overall route is flexible enough to allow for the synthesis of analogues, and should be of great value for further biological investigation of the way that these compounds work. ☐



Synthetic schemes to two natural products (see text).

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OCEAN DRILLING PROGRAM

Evolution of the Japan Sea

Leg 127 and Leg 128 shipboard scientific parties

LEGS 127 and 128 of the Ocean Drilling Program spent the summer and early autumn of 1989 drilling at six sites in the Japan Sea (see figure). The goals of these two expeditions aboard the RV *JOIDES Resolution* were to assess the timing, style and dynamics of the opening of this sea and to decipher its subsequent palaeoceanographic evolution.

The western Pacific region is characterized by a series of marginal basins bordered by many island arcs. The formation of these marginal seas is intimately linked to the subduction of oceanic crust at adjacent deep-sea trenches. This process fuels the volcanic arcs and creates new oceanic crust and marginal seas through sea-floor spreading and faulting in the back-arc areas between the subduction zone and the continent. The Japan Sea is a large back-arc basin with water depths greater than 3,500 m, separated from the adjacent Pacific Ocean by shallow bathymetric sills of less than 150 m depth. The geology and geophysical structure of the Japanese Islands point to the presence of continental crust beneath the Japan arc, which in turn calls for a history involving continental rifting and separation of the arc from mainland Asia through back-arc formation of the Japan Sea — a tectonic scenario thought to be responsible for the evolution of many ancient continental margins.

Sixteen years ago, Leg 31 of the Deep Sea Drilling Project (DSDP) attempted to study the history of the Japan Sea but failed to penetrate the sedimentary cover and sample basement rocks, thus leaving many fundamental questions unanswered. So the highest priority of Legs 127 and 128 was recovery of basement rocks, which should record the earliest phases in the evolution of the sea. Leg 127 drilled four locations in the Yamato and Japan basins (sites 794, 795, 796 and 797), where rocks that represent acoustic basement on seismic reflection profiles were penetrated for the first time and complete overlying sedimentary sections were recovered. Leg 128 followed by drilling at two topographic highs on the sea floor, including site 798 on Oki Ridge which yielded a key Pliocene and Pleistocene palaeoceanographic reference section for the sea and an unusually clear record of explosive volcanism during this period. Site 799 was located in the Kita-Yamato Trough, a sediment-filled graben (rifted valley) which splits the Yamato Rise. This trough is thought to represent a failed rift within a fragment of continental crust that was isolated during initial back-arc spreading. It is thought to be an ideal setting for the formation of volcanigenic massive sulphide deposits, and it represents the first CDP site selected specifically to test ideas about deep-sea ore genesis. Drilling at this site produced a sequence representing the Lower Miocene through to the Holocene, which will enhance our understanding of metalliferous back-arc environments. Leg 128 also returned to site 794 in the northern Yamato Basin to perform two downhole geophysical experiments designed to probe the structure and character of the crust and upper mantle beneath the Japan Sea.

Drilling by Leg 127 at sites 794, 795 and 797 established that the volcanic basement rock encountered at these sites is of Lower Miocene age. Lithological sections representing acoustic basement at site 797 included interlayered coarse turbidite sands and basalt sills and flows, whereas the basement at site 795 was formed from brecciated basaltic lavas. Thus these facies probably explain the thick oceanic crust indicated by seismic velocity profiles beneath the Yamato Basin. The combined drilling of Legs 127 and 128 at site 794 penetrated 190.5 m into a basal series of stacked tholeiitic dolerite and basalt sills and flows representing the early Miocene floor of the northern Yamato Basin. Deep-water microfossils recovered from small sediment sequences intercalated within volcanic rocks at site 794 attest to the rapid subsidence of the Yamato Basin shortly after initial back-arc rifting, a process confirmed by the results from sites 797 and 799. Indeed, all evidence obtained by Legs 127 and 128 indicates that the Yamato Basin was formed by extremely rapid subsidence during the early Miocene (probably before 19 Myr ago) accompanied by intense volcanic activity.

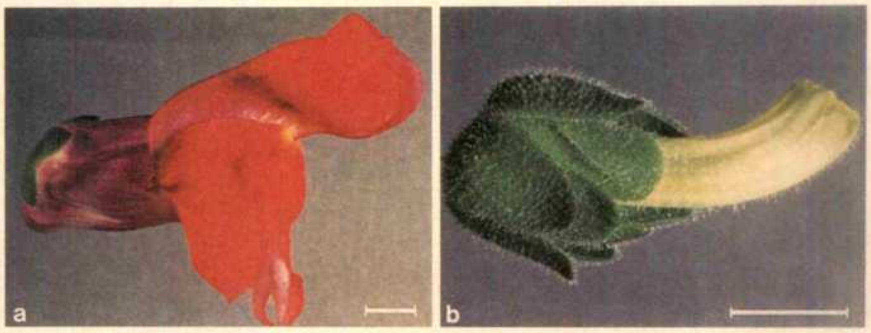
The sedimentary sequences overlying basement rocks at all six sites in the Japan Sea show striking regional similarities and form five distinct lithological associations which record major tectonic and palaeoceanographic changes during evolution of the sea. These include (1) Lower Miocene delta-front sands and siltstones rich in plant debris, which were deposited rapidly during initial back-arc rifting and basin subsidence; (2) widespread Middle Miocene siliceous and carbonate-rich hemipelagic claystones and volcanic tuffs, marking a period when the rate of basin subsidence outpaced the rate of sediment accumulation in the sea; (3) Upper Miocene claystones and porcellanites; (4) Pliocene diatom oozes representing evidence of climatic cooling, upwelling and increasing primary productivity in the sea; and (5) light/dark rhythmic Plio-Pleistocene sediments, reflecting rapid oscillations in the oceanographic behaviour of the Japan Sea from stagnant, near-anoxic conditions to the fully oxic and hyperventilated conditions exemplified by the sea at present. Significantly, the latter cyclic sequences are best developed in the upper Pleistocene sediments which represent the past 400,000 years, during which time basin sills and climatically induced periods of low sea level may have periodically isolated the Japan Sea from the open Pacific.

The relatively recent tectonic emplacement of shallow bathymetric sills or straits separating the Japan Sea from the Pacific Ocean is one manifestation of a convergent plate boundary apparently developing along the eastern margin of the sea, as evidenced by uplifted en echelon ridges, thrust faults and large compressional earthquakes. Leg 127 drilling at site 796 revealed that gravity-driven deposition filling the northwestern Japan Basin stopped, and uplift of the Okushiri Ridge above the basin floor subsequently

Changing the flowers

WITH the report by Sommer *et al.* of the cloning of the snapdragon *deficiens* gene (*EMBO J.* 9, 605–613; 1990), and that by Yanofsky *et al.* of the cloning of the *agamous* gene in *Arabidopsis thaliana* (page 35 of this issue), the study of plant development has taken a big step forward.

The picture, reproduced from the *EMBO Journal*, shows (a) a normal flower of the snapdragon (*Antirrhinum majus*), and (b) one produced by a plant with a mutation at the *deficiens* locus. The petals of the mutant flowers are replaced by a second ring of sepals, the leaf-like organs that form the outer whorl of flowers of this kind, and the stamens are transformed into carpels, which form the tubular structure protruding from the two rings of sepals.



Like *deficiens*, *agamous* is classed as a homeotic gene, because its mutations cause the transformation of one type of flower organ into another. But the effect of *agamous* mutations is quite different from that of *deficiens* mutations: the six stamens of the mutant flowers are replaced by six extra petals, and the carpels are replaced by a whole new flower. The new flower suffers from the same homeotic transformation, so that a repeating pattern of sepals and petals is generated.

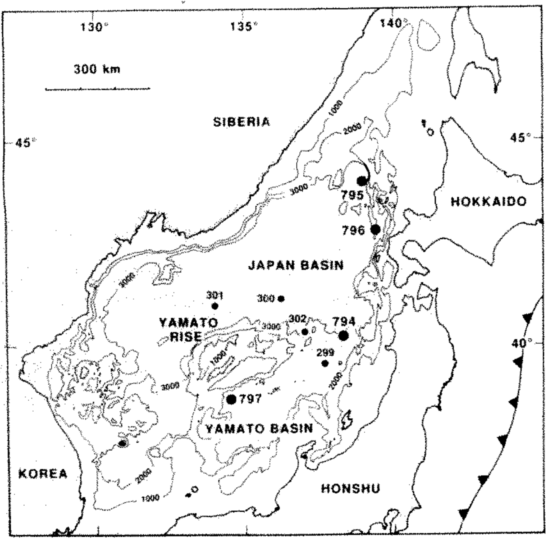
The normal flowers of both snapdragon and *A. thaliana* consist of four concentric whorls of organs: an outer whorl of sepals, followed by whorls of petals, stamens and finally carpels. The mutations that cause transformations of these organs differ in important respects from the better-known homeotic mutations of the fruitfly *Drosophila melanogaster*. The fruitfly homeotic mutations cause discrete transformations of one segment of the fly's body into another, so that a segment is exactly replicated and the segment number is not changed; in the *agamous* mutation, by contrast, the number of petals in the transformed third whorl is six, the same as the number of stamens that would normally be present in this whorl, and not the four that would be expected by a precise reiteration of the second whorl.

The sequences of the *deficiens* and *agamous* protein products show a region of roughly 56 amino acids where they are very similar, both to one another and to two known transcription factors, human serum response factor and the yeast protein MCM1. In the case of serum response factor at least, the conserved region is known to fall within the DNA-binding domain of the protein. So the strong presumption is that flower homeotic gene products form a class of DNA-binding regulatory proteins linked by their similar DNA-binding domains, much as the fruitfly homeotic genes are linked by their 'homeobox' domains.

Geoffrey North

began, in the latest Pliocene (1.8 Myr ago), at the same time that widespread deformation occurred along the entire eastern margin of the Japan Sea. This activity might represent an initial stage in the now continuing compressive destruction of the sea.

Although deep-sea drilling has been very successful in unravelling ocean history by allowing direct study of rocks and sediments in cores, the technique is limited to the upper 1.5 kilometres of ocean crust. Leg 128 carried out two multiship geophysical experiments at site 794 that bridged the gap between direct observations of the upper crust from drilled cores and remotely sensed data on the structure of the deeper crust and upper mantle. The Japan Sea was ideally suited to these experiments because the structure of the deep crust and mantle varies laterally from the Pacific plate (in front of the subduction zone) through the Japanese Island arc to the Japan Sea behind it. These variations reflect the dynamics of the subduction process.



Map of the Japan Sea, showing locations of ODP Leg 127 sites (dots), Leg 128 sites (circled dots) and DSDP sites (triangles). ODP site 794 was occupied during both Legs 127 and 128. Bathymetry in metres.

The first experiment involved installation of a specially designed digital broadband seismometer at 715 m below the sea floor in volcanic basement rock. The emphasis in this experiment was on long-period signals, which probe larger scales and deeper structure. Following an experiment that essentially monitored instrument performance and analysed local crustal structure, the seismometer is now accumulating long-term data on natural earthquakes, which will ultimately be used to create a three-dimensional image of the crust–mantle configuration in this region.

The second experiment comprised active-source electrical measurements designed to give high-vertical-resolution profiles of the electrical resistivity in the crust and upper mantle. Resistivity measurements can yield information on crustal temperatures and the presence of fluids or partial melts. Resistivity observations at site 794 were made by placing an array of electrodes in a drilled hole and having a supporting ship apply an electrical charge to the water column at increasing distances from the drill hole. The results will yield new estimates of electrical resistivity to a depth of 10 km beneath the Japan Sea.

The results as a whole indicate that the marginal basin of the Japan Sea seems to be in a mature stage of evolution despite its recent birth in the late Oligocene to early Miocene. Indeed, the relatively young age of the sea has allowed all of the key elements in the back-arc spreading process to be deduced from samples of sea-floor basalts and sediments, along with the history of the water mass.

Although the data collected during Legs 127 and 128 have yet to be fully analysed, it is clear that the Japan Sea now stands as one of the best documented back-arc basins in the world and provides a potential model for interpreting the rifting and mountain-building processes that characterize many modern and ancient continental-arc settings. ☐

Leg 127. Co-Chief Scientists: Kenneth Pisciotto British Petroleum, UK and Kensaku Tamaki (University of Tokyo). ODP Staff Scientist: James Allan (Texas A&M University). Also, Joanne M. Alexandrovich (Lamont-Doherty Geological Observatory), David A. Barnes (Western Michigan University), Sam Boggs (University of Oregon), Hans-JÜrgen Brumsack (Geochemical Institute, Göttingen), Charlotte A. Brunner (University of Southern Mississippi), Adrian Cramp (University College, South Wales), Laurent Jolivet (Ecole Normale Supérieure, Paris), Orest E. Kawka (Oregon State University), Itaru Koizumi (Osaka University), Shin'ichi Kuramoto (University of Tokyo), Marcus Langseth (Lamont-Doherty Geological Observatory), James McEvoy (University College, North Wales), Jeffrey A. Meredith (Massachusetts Institute of Technology), Karl A. Mertz, Jr. (Miami University), Richard W. Murray (University of California, Berkeley), David C. Nobes (University of Waterloo), Atiur Rahman (University of Utah), Ralph Schaar (Lamont-Doherty Geological Observatory), Kathryn P. Stewart (University of Adelaide), Ryuji Tada (University of Tokyo), Peter Thy (NASA), Luigi Vigliotti (Istituto Di Geologia Marina, Bologna), Lisa D. White (University of California, Santa Cruz), Jobst J.M. Wippern (University of München), Shigeru Yamashita (University of Tokyo).

Leg 128. Co-Chief Scientists: James C. Ingle, Jr (Stanford University) and Kiyoshi Suyehiro (University of Tokyo). ODP Staff Scientist: Marta T. von Breymann (ODP, Texas A&M University). Also: James Bristow (Lamont-Doherty Geological Observatory), Lloyd H. Burckle (Lamont-Doherty Geological Observatory), Jacques Charvet (University of Orléans), Barry A. Cragg (Scottish Marine Biology Association), Peter DeMenocal (Lamont-Doherty Geological Observatory), Robert B. Dunbar (Rice University), Karl B. Follmi (ETH-Center, Zurich), John R. Griffin (University of Nebraska), Kurt A. Grimm (University of California, Santa Cruz), Yozo Hamano (University of Tokyo), Naoshi Hirata (Chiba University), Peter Holler (University of Kiel), Caroline M. Isaacs (US Geological Survey), Michio Kato (Kanazawa University), Richard Kettler (University of Nebraska), Tara Kheradyar (Stanford University), Klaus A.O. Krumsiek (University of Koln), Hsin-Yi Ling (Northern Illinois University), Ryo Matsumoto (University of Tokyo), Jay P. Muza (Florida State University), Ronald J. Parkes (Scottish Marine Biological Association), André Pouclet (University of Orléans), Steven D. Scott (University of Toronto), Rüdiger Stein (Justus-Liebig-Universität) and Anne A. Sturz (Scripps Institute of Oceanography).

DAEDALUS

Sun and air

One of the neatest ways of trapping solar energy is the solar pond. This contains ordinary water, with a layer of denser salt water underneath it. The slow upward diffusion of the salt produces a stable density gradient in the pond; convection cannot occur. Solar radiation penetrates the water and is absorbed by the blackened bottom of the pond. The heat cannot convect upwards; it leaks upwards by conduction only slowly; the bottom of the pond can almost reach boiling point.

In this connection Daedalus recalls the Valley of Death in Java, into which carbon dioxide is steadily released from some subterranean volcanic source. The gas forms a layer on the valley floor. Men and animals who enter the valley (except, possibly, giraffes) are suffocated. But plant life flourishes luxuriantly.

Gases, especially heavy ones, have even lower thermal conductivities than liquids. So, says Daedalus, a gaseous nonconvecting 'solar pond' on the Valley of Death principle should be even more efficient. DREADCO's engineers are now converting an empty reservoir into a prototype. They are roofing the top with a supported web of transparent sheeting, and the bottom with reflective aluminized film. They will then pour carbon dioxide, or some even heavier gas, over the floor of the reservoir. A grid of blackened absorber pipes, supported just off the bottom, will be heated by solar radiation entering from above and reflected from below, while being excellently insulated by the surrounding nonconvective density-stratified gas. It will heat up until convection can just overcome the density gradient against it.

With carbon dioxide, this should happen at 180 °C; sulphur dioxide could give 390 °C and dichlorodifluoromethane could give almost 1,000 °C. Although these limits are unlikely to be reached in practice, the solar gas-pond could clearly generate high-grade heat and power while providing a splendid and ecologically virtuous dumping ground for gaseous industrial nasties. It would, of course, slowly lose efficiency as the stratified gas diffused towards uniform composition. Fortunately, the diffusion-time increases as the square of the mixing distance. A solar gas-pond 40 metres deep would take years to mix completely. Slow removal of the central stratum, while feeding in fresh heavy gas at the bottom and air at the top, could maintain the density gradient indefinitely.

A carbon dioxide solar gas-pond should also act as a wonderful forcing house for tropical vegetation. Its high temperature and insect-suffocating, photosynthesis-encouraging atmosphere should enable coconut palms and breadfruit trees to flourish effortlessly in the bleakest of temperate zones.

David Jones

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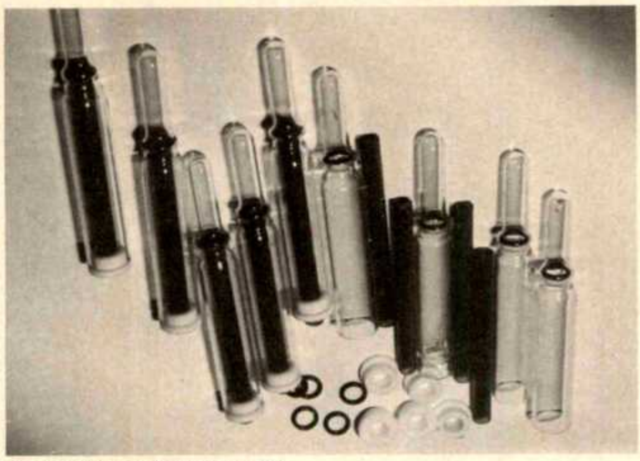

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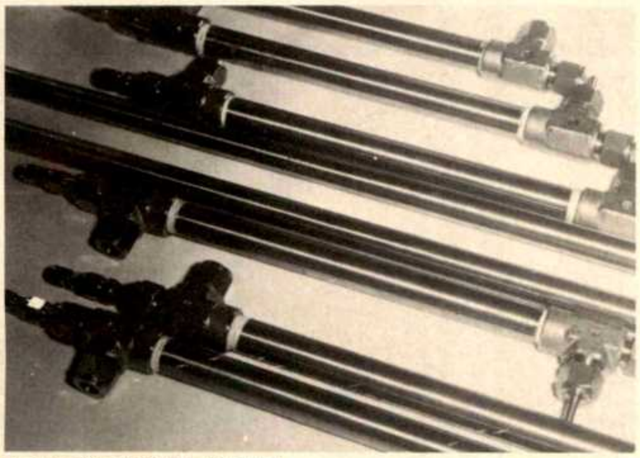

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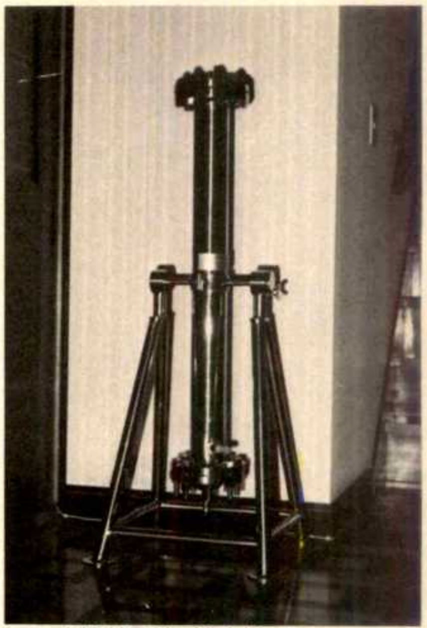

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MICROINJECTION

ELECTROPORATION

COMPARISON

	BAEKONIZATION[1]	*MICROINJECTION*[2]	*ELECTROPORATION*[3]
TRANSFER EFFICIENCY[4]	200 embryos/min	2 embyros/min	200 embryos/min
VIABILITY[5]	90%	70%	<5%
INTEGRATION[6]	40%	25%	0
EXPRESSION[7]	yes	yes	no

[1] Transfer of macromolecules into biological systems by *BAEKON2000* instrument.

[2] Standard microinjection procedure.

[3] Electroporation refers to the treatment of DNA/embryos mixture by a capacitor-based electroporator where both anode and cathode are in direct contact with the sample.

[4] Transfer Efficiency refers to the number of embryos* that can successfully be transferred per unit time.

[5] Viability refers to the survival rate of embryos post-treatment.

[6] Integration refers to the percentage of fish that shows positive hybridization between the DNA from baekonized fish and the DNA probe.

[7] Expression refers to the expression of a growth hormone gene in the transgenic animal.

*Zebrafish

BECAUSE BAEKONIZATION...

1. Facilitates **homogenous** macromolecule transfer into **many** cells, oocytes, fertilized eggs, and spermatogenic cells;
2. Permits transfer to a large group of embryos at the **same** developmental stage;
3. Takes less than 1 minute to transfer millions of cells or hundreds of oocytes or fertilized eggs **simultaneously**;
4. Does **not damage** target cells or **alter** the intracellular environment;
5. Allows **co-** *and* **sequential** transfer of both protein and DNA molecules into target cells, in different combination and permutation, to program gene expression;
6. Results in **expression** and **functioning** of macromolecules inside the baekonized cells, oocytes, or fertilized eggs; and the **integration** of exogenous DNA into the genome.

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Brilliant pebbles won't do

SIR—In their recent Commentary[1], G. Canavan and E. Teller conclude that singlet space-based interceptors (SBI) are ". . . effective in the near-term and mid-term . . ." and that they can be made adequately survivable against achievable Soviet reactions by "hardening, evasion, and decoy in combination . . .". These conclusions stem from the constraints imposed by the authors on the Soviet response. Unaccountably, they assume the Soviets will be able to reduce missile burn time T only to 300 seconds or so. But the Soviets already deploy SS-25 missiles with $T = 180$ seconds, corresponding to an availability of 1.1% for the SBI. Subtracting the SBI "release time" of 50–100 seconds would further reduce the effectiveness of SBI, according to Lt Gen. George L. Monahan's formal response to the US Senate Appropriations Committee on the SDI programme.

Ten warheads deployed on silo-based single-warhead SS-25s within a region a few hundred kilometres or less across would require 10/1.1% = 900 brilliant pebbles to be able to assign even a single interceptor to each missile. As few as 1,000 ICBM warheads would thus need 90,000 brilliant pebbles — a clear demonstration that the defence is not cost-effective at the margin.

The Senate Appropriations Committee went on to ask whether the SDI phase I architecture would be capable of destroying more than a few SS-25s in the boost phase. Monahan answered "No. The phase I SDS (Strategic Defense System) does not provide continuous coverage of the SS-25 deployment region given the available time (SS-25 burn time minus surveillance/command and control timeline)."

If the burn time of a new solid-fuel missile were reduced to $T = 100$ seconds, there would be no intercepts at all. The launch weight of a booster designed to deploy its single warhead in 100 seconds would be about 5% above that of a missile of normal burn time[2].

Canavan and Teller dismiss an even more important and less costly countermeasure: "Attrition during peacetime . . . While anti-satellites would be effective in that role, their use might be viewed as an attack, so the main danger in this case would probably come from ground-based lasers." But the Soviets already launch hundreds of test missiles a year without our viewing them as an "attack". A deployment of SBI could provoke the building of a similar number of Soviet small ASAT weapons, with 40-kilogram homing warheads. Only about 3.5 kilometres per second need be supplied by a two-stage rocket to lift the ASAT to 400-kilometre altitude at precisely the time an SBI is expected at the intercept point. Launching such a small, slow rocket in Kazakhstan could not be regarded as an attack on the United States; it could not reach us, but it could reach our space weapons overflying the Soviet Union.

The key points in making the ASAT clearly cheaper[3,4] and simpler than the brilliant pebbles are that the rocket mass required to launch a 40-kilogram ASAT homing head to intercept altitude is a factor of seven smaller than that required to launch into orbit a 40-kilogram SBI (bearing a 5-kilogram homing head), and the much easier task of an ASAT performing its job within full view of ground-based radar and optical sensors, designators and compound intelligence. This ground-based support would allow the ASAT to identify and destroy manoeuverable, decoyed SBI, or (equally effective) to provoke them to manoeuvere and dispense decoys so that they will be vulnerable weeks or months later.

If helpful, intercepts could be timed so that the intercept takes place in daylight while an observation point on the ground is in the dark. With a phase-I SDI deployment of 4,000 SBI, an SBI would fly within 50 kilometres of the vertical over the ASAT launch site about 20 times an hour, so there are many opportunities for intercept. A deployment of 40-kilogram brilliant pebbles could be defeated at much lower cost and thus would impair rather than add to US security.

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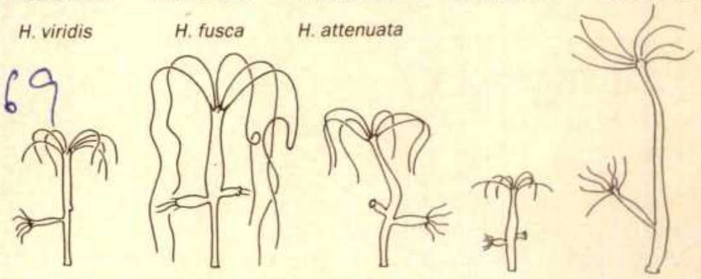
Identity crisis

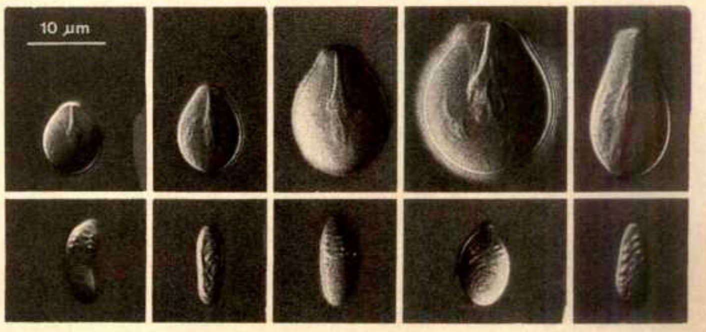
SIR—Hydra are used frequently in research and teaching[1,2], but identifying them is extremely difficult because names have been used inconsistently. Recent work has clarified the names and identities of the five species described in Europe[3-5]. The nematocysts ("stingers") provide the most certain means of identification, and the morphological characters of the body of hydra, as shown in the figure, provide simple guides for identifying the species. *H. viridissima* is immediately identifiable by its green colour, and *H. oligactis*'s identity is revealed by two precocious tentacles on young buds. Identification of the other three species on the basis of morphological criteria is more difficult because the lengths of the tentacles and body vary with culture conditions.

One reason for confusion among names is that the species of hydra most widely used in laboratory research has been

Correct binomial	*H. viridissima* PALLAS 1766	*H. oligactis* PALLAS 1766	*H. vulgaris* PALLAS 1766	*H. circumcincta* SCHULZE 1914	*H. oxycnida* SCHULZE 1914
Recent synonyms	*H. viridis*	*H. fusca*	*H. attenuata*		
Colour	green	brown			
Number of tentacles on young bud	5–6	2	5–6		
Length of body	moderate (5–10 mm)	large (8–15 mm)	moderate (5–10 mm)	small (3–7 mm)	very large (10–20 mm)
Length of tentacles relative to body	½–1	2–4	½–1	½–1	½–1

P 38,669





frequently and incorrectly called *H. attenuata*. This name is no longer considered valid and the species should be known as *Hydra vulgaris* Pallas 1766. Another reason for confusion is that all the European species of hydra have been known by a plethora of names, most of which have been applied to more than one species. The unruly nomenclature has obscured the fact that there are rather few species of hydra and that these are easily distinguishable.

Similar general types of hydra occur on all continents, although the names and the number of species varies. *H. oligactis* and *H. viridissima* are cosmopolitan. In North America, *H. littoralis* and *H. carnea* among others, and in Japan, *H. magnipapillata*, closely resemble *H. vulgaris* of Europe. *H. hymanae* and *H. utahensis* from North America are similar to *H. circumcincta*. Whether some of these are synonyms is not yet known.

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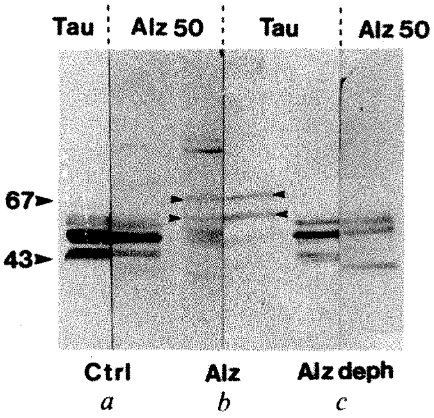
Tau marker?

SIR—The Alz-50 monoclonal antibody recognizes a neuronal antigen, termed A68, exclusively in Alzheimer brain tissue[1]. This antigen, of relative molecular mass 68,000 (68K), is also detected in the cerebrospinal fluid[2] and in the olfactive epithelium from patients with Alzheimer's disease[3], and it has been suggested that Alz-50 might help *in vivo* diagnosis. It was then shown that Alz-50 also cross-reacts with tau proteins[4], the main antigenic component of the paired helical filaments that accumulate in degenerating neurons during Alzheimer's disease, suggesting that A68 might be an aberrant form of tau. We have shown that two tau proteins of M_r 64 and 69K, tau 64 and 69, are specifically detected in brain areas affected by the neurofibrillary degeneration[5]. These proteins arise from an abnormal phosphorylation of tau, as demonstrated by the decrease in their mass after alkaline phosphatase treatment[5]. The crossreactivity of Alz-50 with tau proteins causes us to suspect that Tau 69 and A68 are the same protein.



Binding of Alz-50 and anti-tau on immunoblots of frontal cortex from a control (*a*) and a patient with Alzheimer's disease before (*b*) and after (*c*) alkaline phosphatase treatment. The dephosphorylation was performed as described in ref. 5, and details are available on request from the authors.

Alz-50 was reacted against immunoblotted total protein extracts from different control and Alzheimer's brain areas. These immunoblots reveal that Alz-50 is essentially directed against tau 64 and 69 (see figure) and that no protein other than the pathological tau proteins are detected in this molecular-mass range. The experimental dephosphorylation of tau 64 and 69 results in the formation of 50–60K products, which are still labelled by Alz-50. Immunoblots of two-dimensionally resolved proteins corroborate these findings.

Our results strongly suggest that A68 is an abnormally phosphorylated tau protein. Because the expression of tau 69/A68 is correlated with neurofibrillary degeneration and intellectual impairment[6], Alz-50 marker might be used to help diagnosis of the disease. Moreover, the further study of tau 69/A68 could help track the biochemical dysfunctions that lead to neuronal death and dementia.

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Correction

In the Commentary article "Duesberg, HIV and AIDS" (*Nature* **345**, 659–660; 1990) it is said that homosexuals in the United States have a 900-fold higher risk of developing Kaposi's sarcoma. As stated in the same paragraph, the 900-fold higher incidence referred to HIV-positive homosexuals compared to HIV-negative homosexuals. ☐

Mutagen test

SIR—It is commonly assumed that environmental mutagens are responsible for most mutations in germline and somatic cells, and that mutations arising through endogenous processes such as depurination, deamination, replication error and transposition are comparatively infrequent. With the development of direct sequencing methodology, enough mutational events can be examined to address this issue more directly. Mutagens should produce different mutation patterns in populations exposed to different environments, as each mutagen has its own fingerprint of mutation[1]. By contrast, endogenous processes could produce an essentially invariant pattern of mutation. I find the spectrum of germline mutations from a particular sample to be similar to the spectrum of somatic mutations associated with the p53 and retinoblastoma genes, suggesting that endogenous processes are dominant in these cancers. I propose that tumour-suppressor genes could be useful epidemiological tools for determining the exceptions where environmental mutagens do predominate.

A meaningful comparison of the pattern of mutation requires genes where the observed pattern faithfully reflects the underlying pattern of mutation. From a population-based study of Caucasian families with haemophilia B residing in the midwestern United States and Canada (see table), we deduced[2] that the observed mutations do approximate the underlying pattern (corrected for biases of mutant ascertainment) of recent germline mutations in factor IX. A similar pattern of mutation occurs in Asians[3].

The tumour-suppressor genes offer an opportunity to examine the pattern of somatic mutation *in vivo*, without the artefacts induced by tissue culture. By contrast, dominant oncogenes, such as *ras*, are not good models for examining the pattern of spontaneous somatic mutation because only a few mutations will produce disease. Mutations in the p53 and retinoblastoma genes have recently been published (see table), and the pattern of somatic mutation in colon, brain, breast, and retina is similar to the pattern of germline mutation. Lung tumours are an exception to the general pattern ($P<10^{-4}$); most cases occur in smokers, exposed to enormous amounts of inhaled mutagens.

These data suggest that the underlying

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DISTRIBUTION OF POINT MUTATIONS

	Germline cells			Neoplastic cells		
Mutational type	Factor IX Caucasians*	Factor IX Asians	p53 Colon	p53 Bn/Br	Rb Retina	p53/Rb Lung
Transitions	31	10	11	6	5	0
(Transitions at CpG)	(12)	(6)	(6)	(3)	(3)	(0)
Transversions	5	4	2	0	1	8
Micro-deletions/insertions	2	2	2	0	2	1
Ref.	2	3	4,5	5	6	5–8

*The data approximate the underlying pattern of germline mutation in factor IX. Transitions at CpG are elevated 24-fold relative to other transitions (ref. 2). When the subset of Caucasians with severe disease was compared with the Asian sample, the patterns were essentially identical. The excess of deletions in retina cells reflects the large and highly redundant nature of the retinoblastoma (Rb) protein. Five of the six base substitutions produce nonsense or splice junction defects, implying that the more numerous missense mutations are underrepresented because they generally do not cause disease.

pattern of germline or somatic mutation will commonly be the same. It seems most likely that there is a strong selective pressure for endogenous control of the mutational process, rather than a ubiquitous and prominant mutagen. For germline mutation, the rate may be constrained by opposing evolutionary forces. Too little germline mutation would result in extinction of the species, as the level of variation in the population would be insufficient to adapt to environmental changes. Conversely, more than the requisite amount would increase the morbidity of the species as virtually all disease has a genetic predisposition.

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Sensing reducing gases

SIR—The recent report[1] of a novel thin-film electrochemical cell for energy conversion prompts us to describe some work we have been carrying out using a related concept for the purpose of detecting reducing gases such as hydrogen and carbon monoxide in air.

Our cell is shown schematically in Fig. 1. Its solid components are a porous oxide substrate (polymer substrates, as described in ref. 1 are also useful) and two electrodes of dissimilar metals which are deposited by d.c. sputtering. For an electrolyte we depend entirely on a film of moisture adventitously absorbed from the atmosphere onto the surface of the substrate between the electrodes. With electrodes of platinum and gold, respectively, and the cell exposed to air in the relative humidity range between around 20 and 100% at room temperature a substantial open circuit potential (up to 500 mV) is generated. Operation requires the presence of both oxygen and water vapour.

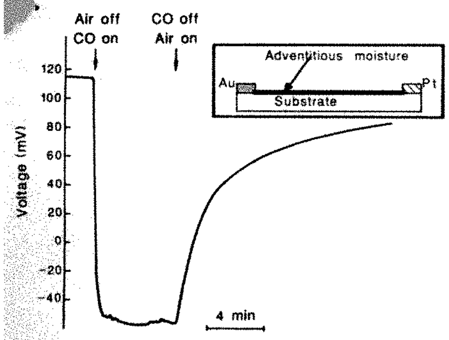

FIG. 1 Voltage response of WO_3 to 1% CO in air. Open-circuit potential from a cell based on a porous pellet of tungsten trioxide with platinum sputtered on to one flat surface and gold on the other. The potential difference was monitored via a Keithley model 614C electrometer with the platinum electrode connected as positive. The atmospheric compositional change between air and air containing 1% carbon monoxide at the points marked gave rise to a change of over 150 mV in the open circuit potential of the cell. Inset: schematic of the thin-film electrolytic cell.

The geometry of the three-phase interface involving a film of adsorbed moisture is ideally suited for sensing gas reactions: changes in open circuit potential result when the cells are exposed to small concentrations in air of a range of gases including hydrogen, carbon monoxide, ammonia, hydrogen sulphide and ethanol vapour (Fig. 1). The open circuit potential change of the cell increases approximately as the log of the concentration of the reducing gas in an air ambient (Fig. 2).

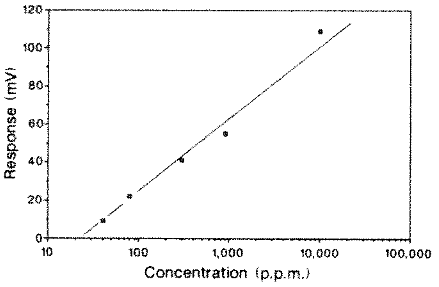

FIG. 2 Variation in the magnitude of the change in potential of a cell based on a porous pellet of tin dioxide, with one electrode platinum and the other gold as a function of the logarithm of the concentration of hydrogen in air.

We believe the mechanisms of response as involving changes in "mixed potentials"[2], which are different on different metals. An electrode at open circuit adopts a potential (mixed potential) such that the rate of all the anodic and cathodic processes balance. In air, in the absence of any other gases, the processes are the reduction of oxygen, and the reduction and oxidation of the metal:

$$\tfrac{1}{2}O_2 + H_2O + 2e^- \rightleftarrows 2OH^- \qquad (1)$$

$$Pt + 2OH^- \rightleftarrows PtO + H_2O + 2e^- \qquad (2)$$

$$Au + 2OH^- \rightleftarrows AuO + H_2O + 2e^- \qquad (3)$$

The balance between reactions (1) and (2) establishes a different potential from that between reactions (1) and (3), hence accounting for the open-circuit potential difference of the cell. The presence of a fuel gas such as CO introduced the possibility of a different reaction:

$$CO + 2OH^- \rightleftarrows CO_2 + H_2O + 2e^- \qquad (4)$$

which we surmise affects the mixed potential predominantly on the platinum electrode rather than on the gold, and hence gives rise to a change in the output of the cell.

As in the case of the thin-film electrolyte fuel cell[1], our device exhibits the convenient attributes of operating at room temperature and functioning without the need to separate the analyte (fuel) gas from the oxidant. The key role of reaction kinetics is also common to both applications.

For sensor applications the cell we have described offers a number of useful features: independence from the need for a reference volume leads to simplicity; room-temperature operation renders the detection of potentially flammable gases such as hydrogen a safer prospect and the open circuit potential in air provides a useful 'condition safe' indicator.

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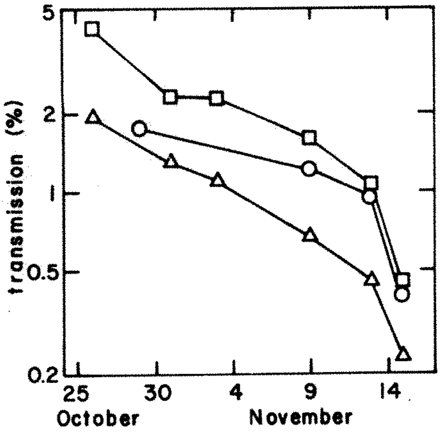
Scientific Correspondence

Scientific Correspondence is intended to provide a forum in which readers may raise points of a scientific character. They need not arise out of anything published in *Nature*. In any case, priority will be given to letters of fewer than 500 words and five references. ☐

Radiation risk

SIR—There is a significant increase of ultraviolet levels in the Antarctic during the presence of the 'ozone hole'[1], and consequently there is concern for Antarctic life forms which may have developed only minimal defences against this radiation[2,3]. We have previously pointed out[4] that the problem is potentially most acute under the vast sea ice cover surrounding Antarctica, for the sea ice is particularly transparent when the ozone hole occurs. Because we did not know the ultraviolet properties of the ice, our argument relied on an extrapolation from data in the visible part of the spectrum. Here we report measurements showing that the transmission of sea ice shows an even larger seasonal variation in the ultraviolet than in the visible spectrum.



Seasonal development of the transmission of sea ice at wavelengths of 300 nm (ultraviolet-B, triangles) 350 nm (ultraviolet-A, squares) and 650 nm (mid-visible, circles).

The experiments were performed on 1.7-m-thick first-year sea ice in McMurdo Sound, using a technique described elsewhere[5]. The measured development of the transmission of the ice at three wavelengths is shown in the figure. All three curves show a substantial decrease during the spring, resulting from the development of a turbid surface layer left behind after brine pockets in the ice drain[6]. Surprisingly, the development is stronger in the ultraviolet than in the visible, for which we do not yet have a satisfactory explanation.

Algae residing near the ice–water interface are the first major biological population to experience the radiation entering the ice. These algae have been estimated to account for up to 30% of the primary food production in the ice covered regions of the southern ocean[7], and they are known to be sensitive to ultraviolet radiation[2]. We have estimated the level of flux falling on these algae as the product of the sea ice transmission of the figure and the surface flux measured at Palmer Station during the 1988 spring[1]. In the latter half of October, the under-ice flux rises as high as 10^{-3} W m^{-2} in a 10-nm band centred on the more damaging ultraviolet-B region, up from about 0.3×10^{-3} W m^{-2} before the appearance of the ozone hole. The 1988 ozone depletion was rather weak, and for the stronger depletions of 1987 and 1989 the October under-ice flux in the same band[8] would rise to about 5×10^{-3} W m^{-2}. Later in the season, the turbid surface layer shields the ice, so that in the height of summer the flux in this band is very much lower, about 5×10^{-5} W m^{-2}. Thus the yearly dose at the base of the ice is overwhelmingly dominated by the October flux, with the consequence that the ice algae will have experienced more than a tenfold increase of ultraviolet-B dose in recent years compared to the pre-1975 levels.

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Cortical maps

SIR—Durbin and Mitchison[1] suggest an explanation for cortical maps and their distortions. If their purpose is to explain the biological conditions for the formation of two-dimensional brain maps, a theoretical description of the lateral interactions between neural cells would require the use of spatio-temporal partial differential equations[2,3]. But to implement powerful artificial 'neural' computing structures (which can form dimensionality-reducing mappings and abstractions), the self-organizing process is better described by computational algorithms that simultaneously update sets of neighbouring neural units[4].

Contrary to what Durbin and Mitchison claim, the equation they use (see their legend to Fig. 2) is not based on Hebb's law of synaptic plasticity; the first term would need a more lengthy justification[5]. Their second term was introduced "to make neighbours have similar receptive fields", but they did not explain what kind of biological adaptive process would account for this effect. This term in fact seems to stem from the physical analogy of stress–strain relations in an elastic ring or net[6]. In the other works cited by Durbin and Mitchison, on the other hand, lateral interaction has been used to activate neighbouring cells to learn from the same input, which is a different and natural assumption.

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DURBIN AND MITCHISON REPLY—As Kohonen points out, self-organizing models have previously been used to simulate cortical maps. We did not intend to suggest the contrary, and we did, in fact, cite Kohonen's book and Swindale's work. However, we should perhaps have spelt out more clearly the ancestry of our simulation. Of course, the main goal of our paper was to consider wiring minimization as an organizing principle for maps of many variables, and to relate this to self-organizing models. We believe this is a new approach to cortical mapping.

We are surprised at Kohonen's rather critical comments about the self-organizing model we used, since it operates in a similar fashion to his own. Both work at a fairly abstract level, which we believe was appropriate for our paper. As he remarks, the first term in our equation, though not directly hebbian, can be derived from Hebb-like interactions. Our second term can be justified in a similar way, by averaging neighbour interactions of the sort he describes; the 'elastic net' analogy is only intended to give intuitive guidance on the way the model works. The resulting equation has the advantage of a cleaner mathematical form which can be analysed in detail[7]. Empirically, it gives results similar to his and other self-organizing models, and in our hands is certainly no less computationally effective.

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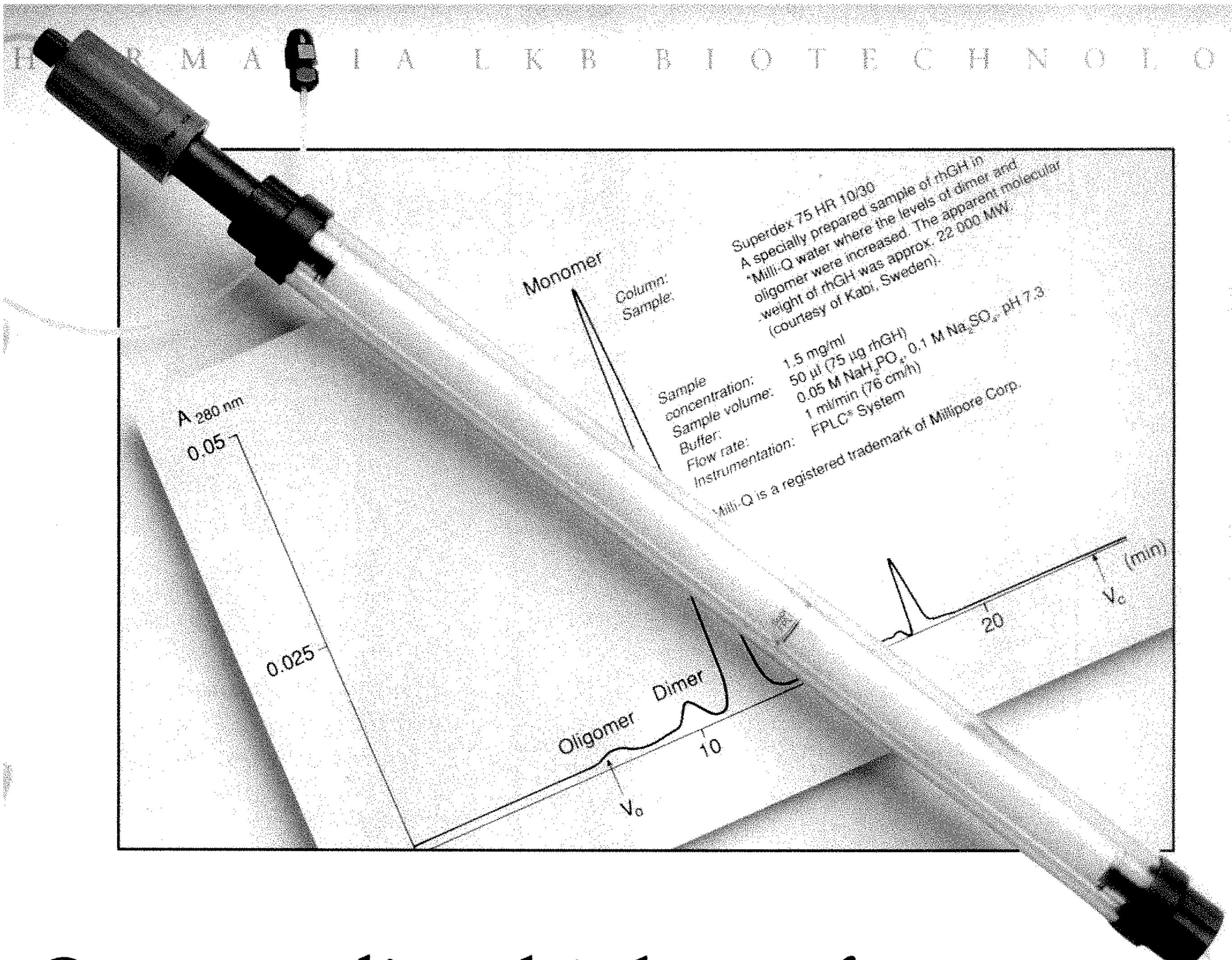

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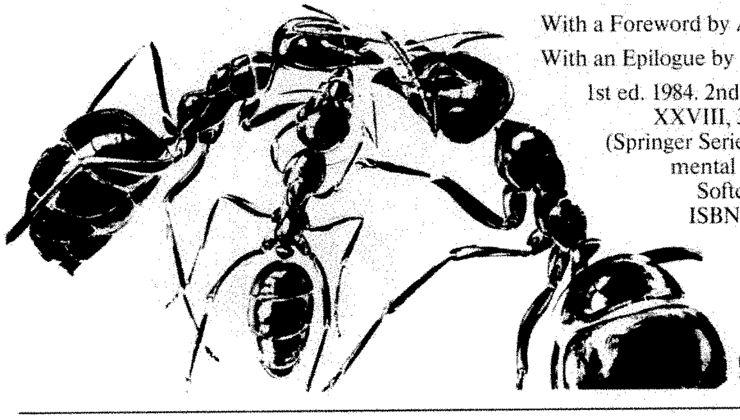

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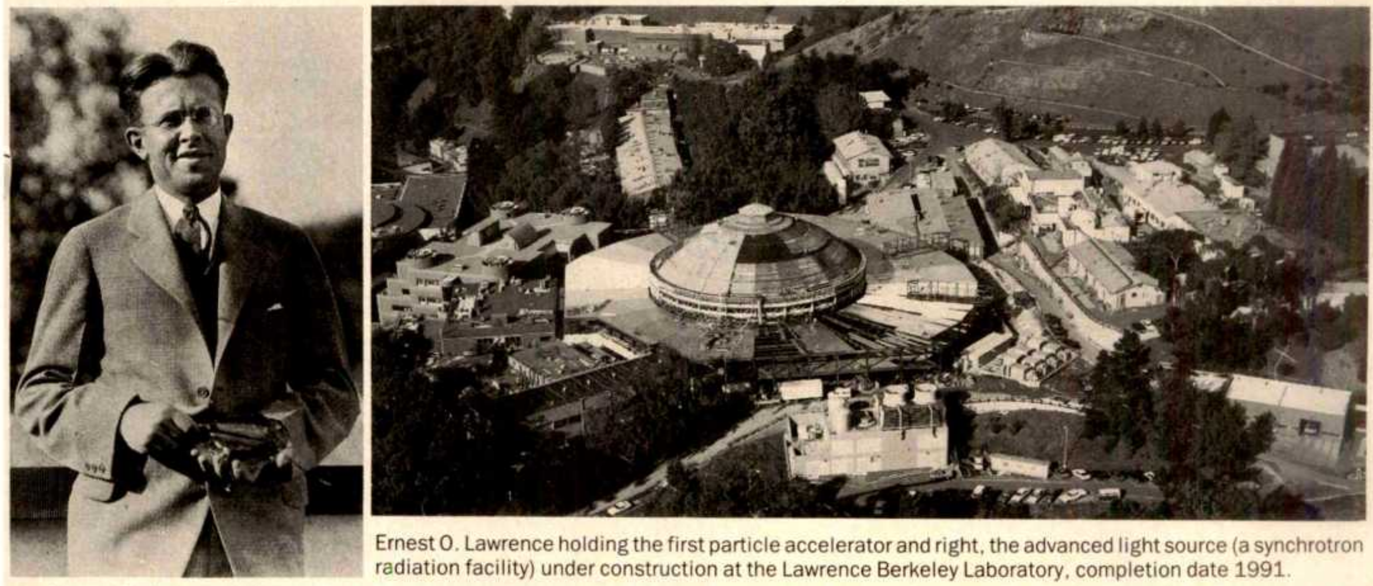
One man and his lab

John Stachel

Lawrence and His Laboratory. A History of the Lawrence Berkeley Laboratory. Vol. 1. By John L. Heilbron and Robert W. Seidel. *University of California Press: 1989. Pp.586. $29.95.*

An understanding of just how high-energy physics reached its present state of apparently limitless demands on the world's physical and financial resources — as well as on the human resources of many physicists — can well begin with a close scrutiny of "the rise of Big Science as it was born in the Rad Lab" (Martin Kamen, *Radiant Science, Dark Politics*). Those of us who are not technological determinists want to get a sense of how the nuclear physicists of the 1930s, through cooperation and conflict with their fellows and with the rest of their social milieu, created the first generation of particle accelerators; and how the result in turn helped to shape the lives and deeds of succeeding generations of particle physicists. We want to get a sense of the alternatives that were faced, the crucial choices that were made — and why — and of potentialities left unexplored as a consequence of these decisions. Heilbron and Seidel have in large measure succeeded in fulfilling these hopes in the first volume of a projected trilogy, which carries the story to 1941, the year of America's entry into World War II.


Lawrence Berkeley Laboratory

Ernest O. Lawrence holding the first particle accelerator and right, the advanced light source (a synchrotron radiation facility) under construction at the Lawrence Berkeley Laboratory, completion date 1991.

We watch Lawrence himself pick up the basic idea for the cyclotron, which had previously occurred to many people, and develop it into a working machine, using his enthusiasm, energy and considerable skills as an organizer to build up a remarkable group of doctoral students and postdoctoral co-workers — his "boys" in the typical male-bonding language they all used. Heilbron and Seidel emphasize that, if Lawrence "himself had not uncovered much new about the nucleus", his "notable contribution" was "the cyclotron laboratory" (p. 489). As Livingston, one of the first and most notable of the boys — and one of the first to break free of Lawrence and his lab — put it: "His optimistic and inspirational attitude was what convinced me it was worth working on" (p. 486).

Yet Lawrence himself was changed by the course of his own success. The process of reaching for ever-higher energies and ever-bigger machines seems to have become an end in itself for him, an imperative only loosely coupled to uses to which the beams emerging from his machines could be put. "Lawrence sets goals expressible not in terms of progress in physics, but in terms of increases in decimals" (p. 480). While Lawrence and his brother John, a physician who worked on medical applications of the newly available particle beams, were undoubtedly sincere in their efforts to benefit humanity ("It means a great deal more to civilization . . . to find a new radiation or a new substance that will cure disease than it would to discover a new supernova", Ernest said in 1940), there was just as undoubtedly an element of manipulation in the way these prospects were dangled before prospective donors — and the general public — as a means of obtaining the financial support that became more and more necessary to the realization of Lawrence's ever more ambitious dreams. He was forced by the logic of his position as an experimentalist who had staked his whole career on the building of ever bigger and more elaborately instrumented cyclotrons to become the intimate of university administrators, foundation nabobs and influential industrialists. His induction into the Bohemian club, a retreat for the rich and powerful of San Francisco and their university confrères, fairly early in his career at Berkeley can be taken as an early indication of his success. The award of almost one-and-a-half million dollars by the Rockefeller Foundation and the trustees of the University of California in 1939 marked the culmination of his efforts: "The munificent grant represented many things: dollars to be sure, but also the affection, respect and confidence in which Lawrence's fellow physicists and prominent men of business held him" (p. 482).

For the first time, funding for nuclear physics began to rival that for an older American mania for the super in science, big telescopes; the parallel with the 200-inch telescope was explicit in the funding hype for the cyclotron — "the Palomar of the vanishing small". By this time, almost all the main ingredients for contemporary big science were in one place, except one: large-scale financial support from the federal government. As the authors indicate in the last chapter, World War II was to provide that ingredient.

The change in Lawrence was not without its costs to the laboratory and to the man. A veteran of the lab in the 1930s, Morton Kamen, reports that Lawrence "was almost always on the road by 1939. The early magic of the Rad Lab had largely vanished", to be replaced by "a new, less personal ambience, unpleasant in its demands for aggrandizement". Another ingredient had been added to the recipe for a modern high-energy physics facility. Kamen connects Lawrence's "untimely death at the age of fifty-seven of [chronic] ulcerative colitis" with the inflexible demands that he came to make on his family, his lab and himself. There are few hints of this darker side of the Lawrence story in the present volume, although we may anticipate more in the sequel.

Lawrence's ambitions were not only intensive — the biggest machine possible — but also extensive: "There should be a cyclotron lab in every university center" he said in 1938. He generously exported the know-how — and the people who knew how — to physics centres in the United States and abroad. By 1940, "there were 22 cyclotrons completed or under construction in the United States" (p. 308), hardly enough to satisfy Karl K. Darrow, the Polonius of American physics, who opined: "The country may need a thousand cyclotrons" (p. 311).

One gets a sense of lost potentialities for other modes of development of nuclear physics from the initial resistance to the spread of the cyclotron by the best of the British and finest of the French nuclear physicists. Lawrence was often reproached for careless work and unjustified claims in the early years of the lab, such as his notorious argument for the supposed instability of the deuteron. Heilbron and Seidel make a rather harsh assessment: "The frequency with which the laboratory had to retract published results had declined since Lawrence had gone full time into fund raising and administration" (p. 385). In any case, it is certainly a "disagreeable fact that no major discovery had yet been made [by 1940] in any cyclotron laboratory".

The great discoveries in nuclear physics in the 1930s were made by small groups, getting the most out of small accelerators, careful chemical analyses and free-of-charge cosmic rays. No wonder Rutherford and Chadwick, Cockcroft and Joliot-Curie initially resisted the call from California. In another preview of post-war things to come, the first foreign converts to the Berkeley gospel were the Japanese, based on "a conviction on the part of government and industry that excellence in Western science was essential to Japan's place in the sun" (p. 317).

The need to move to higher energies than conventional methods could reach impressed itself on British, French and German savants in the late 1930s; by this time, the initial quirks that had made cyclotronics more of a dark art than a working technology had been resolved, and Lawrence's machine stood ready as the only technique able to meet the need. Something else went along with these exports: "The fascination with hardware and the subordination of the individual to the group that characterized Berkeley by the late 1930s were to spread from the accelerator laboratories to other parts of physics and from the United States to the rest of the world" (p. 351).

Heilbron and Seidel, with Bruce Wheaton, have elsewhere described the story of Lawrence and his laboratory as "a strong interaction between science and society" ("Lawrence Berkeley Laboratory 1931–81" *CERN Courier*, October 1981). Unfortunately, the social context they depict is limited largely to Lawrence's relationships with the powers-that-be. Theirs is a history from the top down, which is certainly an important part of the story. As Raymond Williams observed in another context: "A need which corresponds to the priorities of the real decision-making groups will, obviously, more quickly attract the investment of resources and the official permission, approval or encouragement on which a working technology . . . depends."

But if one is to understand the choices Lawrence made in the 1930s, as compared with those made by Berkeley's other rising physics superstar J. Robert Oppenheimer (the often-made contrast between the two is also made in this book), one must tell some of the history of the 1930s from the bottom up. It was a time of severe economic crisis in the United States, but also a time of great hope. President Roosevelt spoke of "one third of a nation ill-housed, ill-clad, ill-nourished", and of the need to curb the power of the trusts if the country was to be put back on its feet. A wave of protest by the unemployed and of trade-union organization by the employed swept the nation, cresting in California. To understand the California of the time one must evoke not only the Federal Telegraph Company and the Research Corporation, as do the authors, but also the general strike in San Francisco and the efforts to organize migrant farm workers, immortalized in John Steinbeck's *Grapes of Wrath*.

The impact of such events and struggles, which did not leave their own plight unaffected, moved a considerable number of US scientists into political action in broad sympathy with the growing movement to organize labour in factory and field — and laboratory. "Suddenly, prominent scientists were in the forefront of both the antifascist and social reform movements . . . A small though vocal and influential portion of the scientific community became radicalized", reports Peter Kuznick in his recent account of this movement (*Beyond the Laboratory*, University of Chicago Press, 1989). It will not do to say, as do the authors, "Lawrence trusted that the world would muddle through without requiring his attention", while "Oppenheimer did not allow the world to get on without his help" (p. 255). Theirs were political decisions, made in the context of national and international conflicts, the outcome of which were to decide the fate of a generation.

It must be added that the authors' aloof and ironic style, while adding considerably to the amusement of the reader, does not serve well the goal of a deeper understanding of the personalities of Lawrence or the other central figures in the drama. Instead of patronization, for example, one would have wanted more help in understanding what many of Oppenheimer's students — and students of his career — consider his finest hour as a human being and social figure. Lawrence also made his decisions. His biographer reports that: "He warned his people . . . 'If anyone wants to write letters to editors and that sort of thing, he should get out of science and get a job on a paper'" (H. Childs, *An American Genius*, p. 267). The authors' discuss quite frankly Lawrence's anti-semitism, of the conventional midwestern variety typical of his generation, and its repercussions in the lab. They do not mention that: "Though he preferred not to have women in the laboratory at first", presumably because of similar conventional prejudices, "a few were eventually accepted" (Childs). Their own attitude towards women is not unobjectionable: in their text, Lawrence's wife is always "Molly", Lawrence is never "Ernest". Their account of the courtship of lab secretaries (all female) by lab scientists (all male) is reminiscent of anthropological accounts of the exchange of women as a means of male tribal bonding (p. 247).

This long book is not without its *longuers*: much of its length can be attributed to the tendency of the authors to follow their material, however far it leads them from Lawrence and his lab: in some measure, they have written a history of experimental nuclear physics in the 1930s. It would have been helpful if the authors had provided an introductory overview of the entire history of the lab; that they have not done so is surprising since they, together with their former collaborator, Bruce Wheaton, have written two such surveys that carry the story through the seventies (one is cited above; the other, published by the lab, has the same title as this book). Even more surprisingly, neither survey is cited in the extensive bibliography here.

The publisher must be commended for the unusually low price of the book, which certainly justifies its computer-driven look. But something has gone seriously wrong with the index, which is a disaster area. An hour's effort turned up nearly 100 missing page references, and about 40 missing names of people and institutions. A more systematic search of one entry (Szilard) turned up about as many missing references as there are in the index. □

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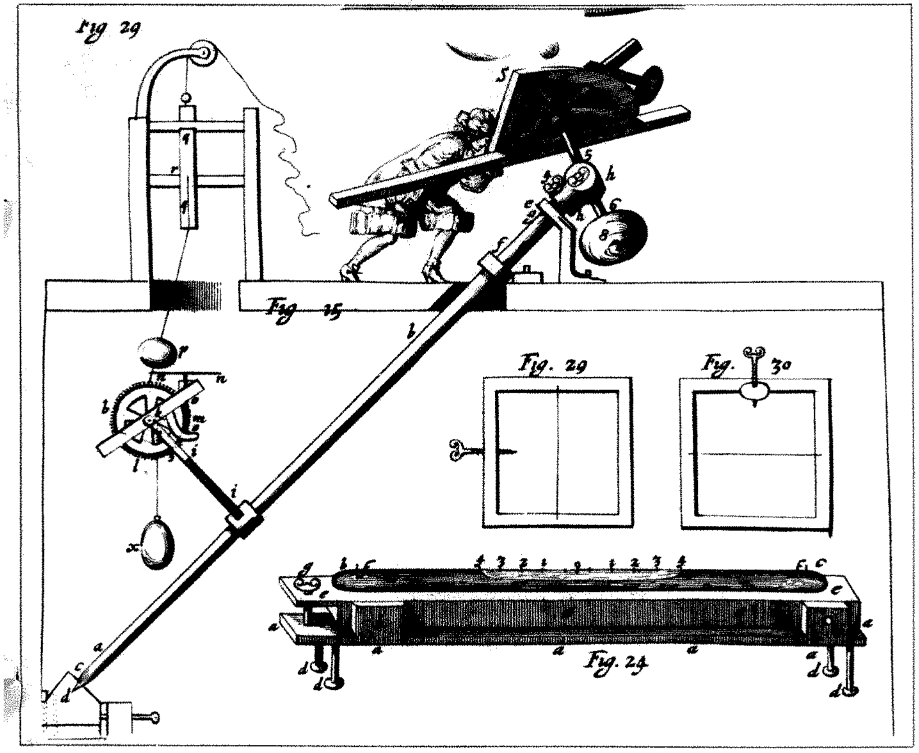
Serious science

Willem D. Hackmann

Robert Hooke: New Studies. Edited by Michael Hunter and Simon Schaffer. *Boydell & Brewer: 1989. Pp. 310. £39.50, $75.*

HOOKE — inventor, architect, surveyor of the city of London and man of science — was one of the most brilliant English scientists in the seventeenth century, eclipsed only by Newton. His mechanical genius was surpassed by no one. Yet in many respects he is still an elusive character, undeservedly better known to the history of science fraternity than to the general public. No portrait of him has survived, although we do have a detailed pen drawing by his biographer Richard Waller: ". . . in person but despicable, being crooked and low in stature, and as he grew older more and more deformed. He was always very pale and lean, and latterly nothing but skin and bone, with a meagre aspect, his eyes grey and full, with a sharp, ingenious look whilst younger. . . He went stooping and very fast, having but a light body to carry, and a great deal of spirits and activity, especially in his youth. . . His temper was melancholy, mistrustful, and jealous, which more increased upon him with his years."



Hooke's equitorial quadrant, with its conical pendulum driving clock. Could the diminutive figure pushing the quadrant be Hooke himself? From Hooke's *Animadversions on the First Part of the Machina Coelestis* (London, 1674).

Hooke became notorious in his lifetime for the many bitter controversies and priority disputes with fellow scientists, notably with Newton, over the theory of colours (1672) and the priority of the law of gravitation (1686), and with Christiaan Huygens over the spring regulator (1674). He also fell out with the Astronomer Royal John Flamsteed, the Danish astronomer Johannes Hevelius, and with a bevy of Royal Society fellows: Sir John Cutler, Henry Oldenburg, Richard Waller, John Woodward and Hans Sloane, to name but the most prominent. Steve Shapin points out in his perceptive chapter in the present volume that some of these episodes might not have resulted from Hooke's cantankerous nature but rather from the ambivalent place he occupied in the *milieu* of seventeenth century science: not quite a 'gentleman scientist' like Robert Boyle, nor a lowly artisan (Hooke styled himself a 'master of technicians'), but someone in a dependent position, an employee who had to survive on patronage. It may not be insignificant that there is no record of him ever having crossed swords with that great patron of science, Robert Boyle, with whom Hooke started his scientific career and who obtained for him the post of Curator of Experiments at the Royal Society. As Shapin concludes, Hooke's pattern of behaviour was not considered exceptional among contemporary tradesmen who had to live from the product of their wits.

This multi-authored volume on different aspects of Hooke yet again illustrates the versatility of the man's genius. What emerges are two fundamental strands of Hooke's work: his enthusiasm for mechanisms (what Richard Waller called "his first and last Mistress"), and his need to underpin his instrumental view of nature by his scientific method. In the opening chapter, Jim Bennett deals with Hooke's instruments for astronomy and navigation and their common concern with angular measurement. The telescope was scarcely regarded as an astronomical instrument before the application of the eyepiece micrometer, championed by Hooke in the early 1660s. A keystone of Hooke's science was his confidence in the potential for instrumental advance as expressed in his controversy with Hevelius, and the development of his novel equitorial quadrant with its conical pendulum driven clock.

A. D. C. Simpson presents an extraordinary detailed analysis of Hooke's concern with practical optics, highlighting the complicated relations between Hooke and the London optical instrument-making trade, and also between Hooke and his colleagues at the Royal Society. Michael Wright has a particular affinity with Hooke because he is a skilled instrument maker. He not only analyses Hooke's attempts to produce a clock for determining longitude at sea, he actually sets about reconstructing the device in brass. David Oldroyd tackles Hooke's enduring interest in geology.

With John Harwood's essay, the book moves from instrumentation to application of the devices, in this case to the strategic use of pictures produced with the microscope to make the new scientific philosophy generally accepted — what Harwood calls the "visual rhetoric" of Hooke's science. John Hendry examines Hooke's scientific method. Hooke not only used instruments to aid the imperfect senses (calling experimentation the "sixth sense" in scientific endeavour), but also as metaphors for describing natural processes. An important aspect highlighted by Hendry is that Hooke's mechanical philosophy cannot be so easily characterized but contains elements of earlier notions of natural magic. Patri Pugliese describes the real contribution Hooke made to Newton's formulation of his inverse-square law of universal gravitation. It is apparent from the ensuing priority dispute that Hooke never appreciated the full magnitude of the step from his ideas to Newton's achievement.

Light relief is supplied by Lucinda Beier's tale of Hooke's determined search for good health, based on his *Diary*. He collected medical recipes (folk remedies and scientific medications) in the same manner as schoolboys collect stamps, reporting on cures such as the use of the blood of a black cat to cure chilblains. Hooke treated his body like a chemical laboratory, testing out all sorts of medicines, such as different types of 'vomits' to purge the system. He was the great medicine-taker of the seventeenth century. Beier teaches us a great deal about contemporary medical practices and attitudes.

What could have been a somewhat dispersive book is ably held together by the introduction. And as its conclusion is a transcript of an hitherto unpublished list of Hooke's possessions at his death discovered in the Public Record Office. Among his dilapidated chattels a chest was found containing £8000 in new and old money, gold and silver. Richard Waller was moved to "a melancholy Reflexion" of how much good this could have done "for the promoting of Learning" if Hooke had only left a will. Instead the money passed on to an illiterate distant relative. Even so, Hooke emerges from this book as the figure that turned the Royal Society into a serious scientific institution. ☐

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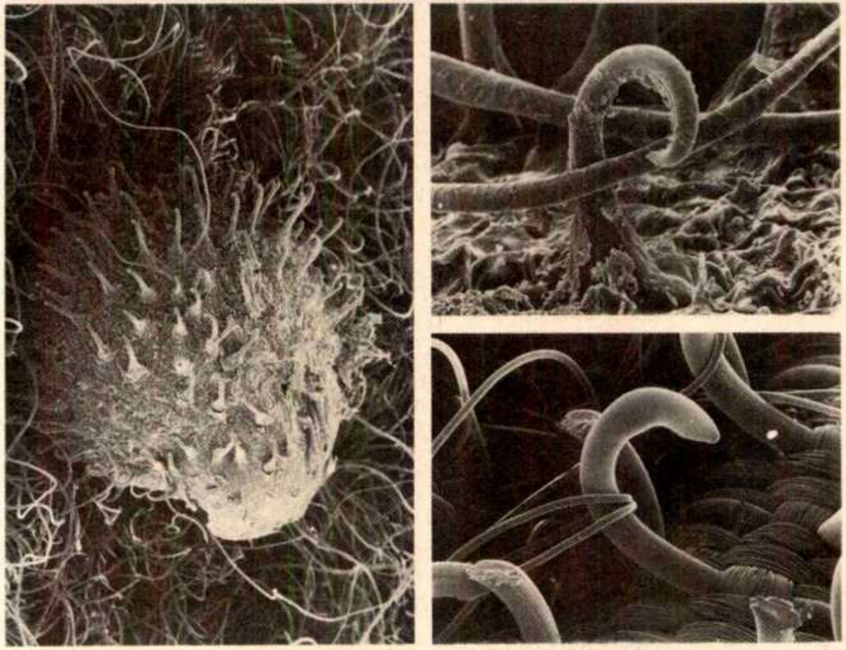

Imitating nature — the fruit of the goosegrass (left) hooked onto a woollen sweater provides the model for Velcro, a tough, manufactured nylon 'hook and loop' fastener. The top right micrograph depicts a goosegrass hook with a single snared wool fibre. Bottom right, the synthetic equivalent, a Velcro hook woven into a fine, flexible nylon base material. Micrographs reproduced from the new edition of *Under the Microscope* by J. Burgess, M. Marten and R. Taylor. Cambridge University Press, £12.95, $19.95.

Tribal tribute

Len Goodwin

Laughing Death. The Untold Story of Kuru. By Vincent Zigas. *Humana: 1990. Pp. 315. $24.95, £29.50.*

VINCENT Zigas, who died two years ago, was born in Germany. He had served as a medical officer in Hitler's army and in 1950, in search of a new life, took Australian citizenship and was posted as a bush doctor to the highlands of New Guinea. He fell deeply in love with the island — its steep mountains, hot, wet forests and, above all, its stone-age people — this is his second autobiographical book about his "green dwelling". Conditions when he arrived were primitive; disease and death were all around. Zigas struggled to understand the native languages, prejudices, taboos and intricate ways of thought and behaviour so that he could improve health and hygiene.

When he went to work in the Okapa district among the Fore people, a distinctive tribe with its own traditions and cannibalistic rituals, Zigas became intrigued with kuru, a neurological disorder attributed to sorcery. Women were more commonly affected than men; the condition struck suddenly, advanced inexorably, causing tremor, ataxia and inane laughter, and was always fatal in about a year. It was not impossible that the strong local belief in sorcery could have caused hysterical behaviour patterns of this kind. As Zigas says, "the native lives in a world in which there is normally very little difference between reality and unreality . . . belief in magic and the supernatural provides ideal soil for the growth of acute suggestibility". But he was not satisfied; he suspected an infectious cause and pleaded with the Australian authorities to back up his investigations. Because of his special relationship with the people, who liked and trusted him, he was able to collect patients for visiting clinicians to examine and blood, urine and post mortem samples for Macfarlane Burnet's scientists in Melbourne.

Nothing much came of Zigas's studies until a visiting virologist from the United States, Carleton Gajdusek, also became fascinated by kuru, avidly collecting case-histories, data and samples for analysis. According to Zigas, who had a big chip on his shoulder about being a 'naturalized' Australian and bossed by those who had never worked in the field and who despised the natives, Gajdusek's activity was resented by the Australian medical hierarchy. The establishment tried to bar Gadjusek and, in the mistaken belief that kuru was a genetic disease, to isolate the Fore people and prevent their movement to other parts of the country. Fortunately, this plan did not succeed.

The suspicion that kuru might be caused by an infectious agent was reinforced by the discovery that scrapie, a disease with similar neurological effects and pathology affecting sheep, could be transmitted to other animals and was caused by a 'slow' virus. Eventually, Gajdusek succeeded in transmitting kuru from human brains to chimpanzees and was awarded a Nobel prize. Transmission in the field was shown to be associated with cannibalism, the women of the tribe preparing the brains of the dead for the ritual feast probably becoming infected through abrasions of the skin.

With the spread of education and the end of cannibalism, kuru has now almost disappeared. But it was the first of the human encephalopathies shown to be transmissible, and this opened the field for the investigation of a whole series of unconventional, very resistant, non-immunogenic viruses and their relation to spongy degenerative diseases of the central nervous system. The lesson taught by kuru, human immunodeficiency virus and, most recently, bovine spongiform encephalopathy seems to be that we transgress the old rules of behaviour at our peril.

This is a not a book for the squeamish — bloody stumps alive with maggots, dying infants, rotting corpses, faeces, pus and every kind of secretion and emission are enthusiastically described. Zigas also tells the story of how, on one occasion, he narrowly missed having a kuru brain cooked and served for his own dinner.

Gajdusek could not have succeeded without him. In an affectionate tribute in the foreward to the book, Gajdusek likens Zigas to a Don Quixote or Danny Kaye in appearance, speech, movements and actions. He disagrees with many of Zigas's 'facts' and judgements, and his "uncertain verdict" on the book is that "he has written the most remarkable work in existence of abstract expressionistic ironical parody, his own style of biography" — and that sounds fair. ☐

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Late Cenozoic uplift of mountain ranges and global climate change: chicken or egg?

Peter Molnar & Philip England

The high altitudes of most mountain ranges have commonly been ascribed to late Cenozoic uplift, without reference to when crustal thickening and other tectonic processes occurred. Deep incision and recent denudation of these mountain ranges, abundant late Cenozoic coarse sediment near them, and palaeobotanical evidence for warmer climates, where high mountain climates today are relatively cold, have traditionally been interpreted as evidence for recent uplift. An alternative cause of these phenomena is late Cenozoic global climate change: towards lower temperatures, increased alpine glaciation, a stormier climate, and perturbations to humidity, vegetative cover and precipitation.

NOT long after continental glaciation had been recognized, Dana[1] linked it to a late Cenozoic rise of mountain ranges. Over the next 100 years, several eminent Earth scientists[2-7] supported and developed this idea, but in all cases the main logical link remained the temporal correlation of mountain building with the onset of glaciation. Then, when geologists discovered more than one Pleistocene glaciation, the significance of this correlation temporarily lost favour.

Now, with Pleistocene glaciations recognized as a consequence of variations in the Earth's orbit[8] superimposed on a long-term global cooling throughout the Cenozoic era, attention has again been directed to the association of this cooling with regional elevation changes[9,10]. Uplift of large mid-latitude terrains could effect a global cooling by three different physical mechanisms. First, increased elevations at temperate latitudes could extend the duration of winter, and with increased durations of snow cover, the albedo should increase[11]. Second, the increase in elevation of large regions should profoundly affect the circulation of the atmosphere, and experiments with general circulation models suggest that the presence of high terrains in Central Asia and western North America would lead to lower global temperatures than if these areas were low[12]. These experiments also match a number of observed regional climate differences that are not obtained in such experiments on landscapes with little or no high terrain[13]. Finally, chemical weathering, enhanced by the increased exposure of minerals eroded rapidly from cold high altitudes and transported to warmer, moister low elevations, would absorb more carbon dioxide from the atmosphere, thus decreasing the natural 'greenhouse effect'[14]. In view of the simple logic of these physical mechanisms, and the impressive correlations of calculated with existing regional climate differences[13], the weak link in the argument relating climate change to late Cenozoic mountain building becomes the inference that mountain ranges and high plateaux rose in late Cenozoic time.

In what follows, we argue that most of the evidence used to infer late Cenozoic uplift could, in large part, be a consequence of the very climate changes that this supposed uplift is thought to have caused. Undoubtedly, some regions are higher today than they were 2 or 10 Myr ago, but many may simply appear to be higher because the geological effects of late Cenozoic climate change could cause the same changes in the landscape and fossil record as regional uplift would.

The Cenozoic climate record

Both palaeobotanical data[15] and oxygen isotopes[16-18] indicate

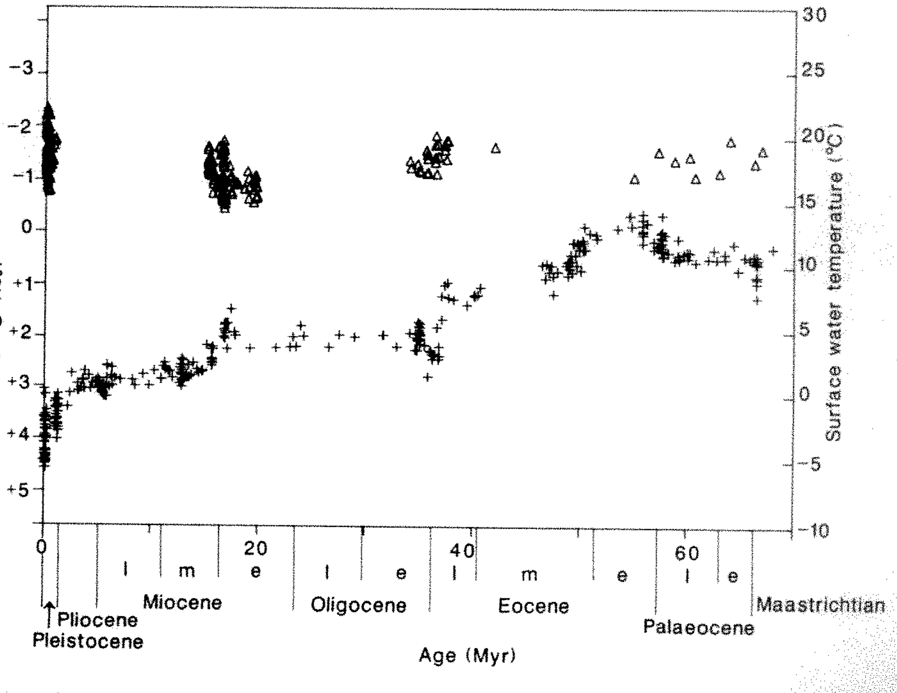

FIG. 1 Oxygen isotope records (and inferred temperatures) for the past 70 Myr from low-latitude surface waters of the Pacific (△) and from ocean deep waters as observed in the South Atlantic (+). Because ocean deep waters are formed at high latitude, the lower curve can be taken as representing the isotope composition of high-latitude surface water. The temperature scale is valid only in the absence of an Antarctic ice sheet—roughly up to mid-Miocene time. Including a correction for the effect of the ice sheet would raise more recent temperatures by ~3 °C. (Figure courtesy of N. J. Shackleton, modified after ref. 17.)

a monotonic, but not steady, cooling of the Earth over the past 50 Myr. Abrupt decreases in temperature occurred near the Eocene/Oligocene boundary[15-21] (~36 Myr ago), at ~15 Myr in the Miocene epoch[16,17,22], and at ~2.5 Myr near the end of the Pliocene epoch[16-18], when continental glaciation in the Northern Hemisphere began[23]. Moreover, the decrease in temperature has been much greater at high than at low latitudes[15,18,24]. For example, oxygen isotopes from planktonic foraminifera deposited at sub-Antarctic latitudes indicate a decrease in surface water temperatures of ~12 °C since late Eocene time[16,17], but those from equatorial latitudes indicate hardly any change in such temperatures[18,24] (see also Fig. 1). Similarly, a comparison of late Quaternary and present-day elevation ranges of plants growing in the equatorial Andes indicates a temperature difference of ~4.5 °C (ref. 25), but oxygen isotopes from cores in Antarctic ice of the same age imply temperatures 11 °C lower than today[26]. The resulting increase in latitudinal temperature gradient is important because it promotes an increased transport of water vapour and latent heat from low to high latitudes[27], and therefore a stormier climate.

Definitions of uplift

The geological literature is much confused by an inconsistent definition of the word uplift[28]. Uplift is a vector opposite to the gravity vector, and a meaningful definition must specify both a reference frame and an object that moves. The most useful reference frames are the geoid (sea level corrected for eustatic changes) and the Earth's surface; the objects that move are either particular rocks or, again, the Earth's surface. In the physical processes linking elevation and climate change[11-13], what matters most is the uplift of the Earth's surface relative to sea level, which is the change in mean elevation. This cannot be measured directly and must be inferred indirectly (see below).

The uplift of rocks relative to the local Earth surface, as measured using geochronology and petrological geobarometers and geothermometers, can be called 'exhumation', and is equal to the thickness of rock removed from the Earth's surface[28,29]. By isostasy (Archimedes' principle applied to light crust overlying heavier mantle), the removal of a layer of crust of thickness ΔT and density ρ_c, compensated by a crustal root in a mantle of density ρ_m, results in a lowering of the surface of $\Delta T\ (\rho_m - \rho_c)/\rho_m \approx \Delta T/6$ (Fig. 2*a*). Thus, the isostatic response to rapid erosion of a region of gentle relief and mean elevation h could transform it into one with peaks at heights of $2h$ (or higher), with valleys near sea level, but with a *lower* mean elevation of only $0.83h$ (Fig. 2*b*).

The uplift of rocks relative to sea level is the sum of the uplift of the Earth's surface and exhumation[28]. As an example, the heights of marine terraces above sea level (corrected for eustatic

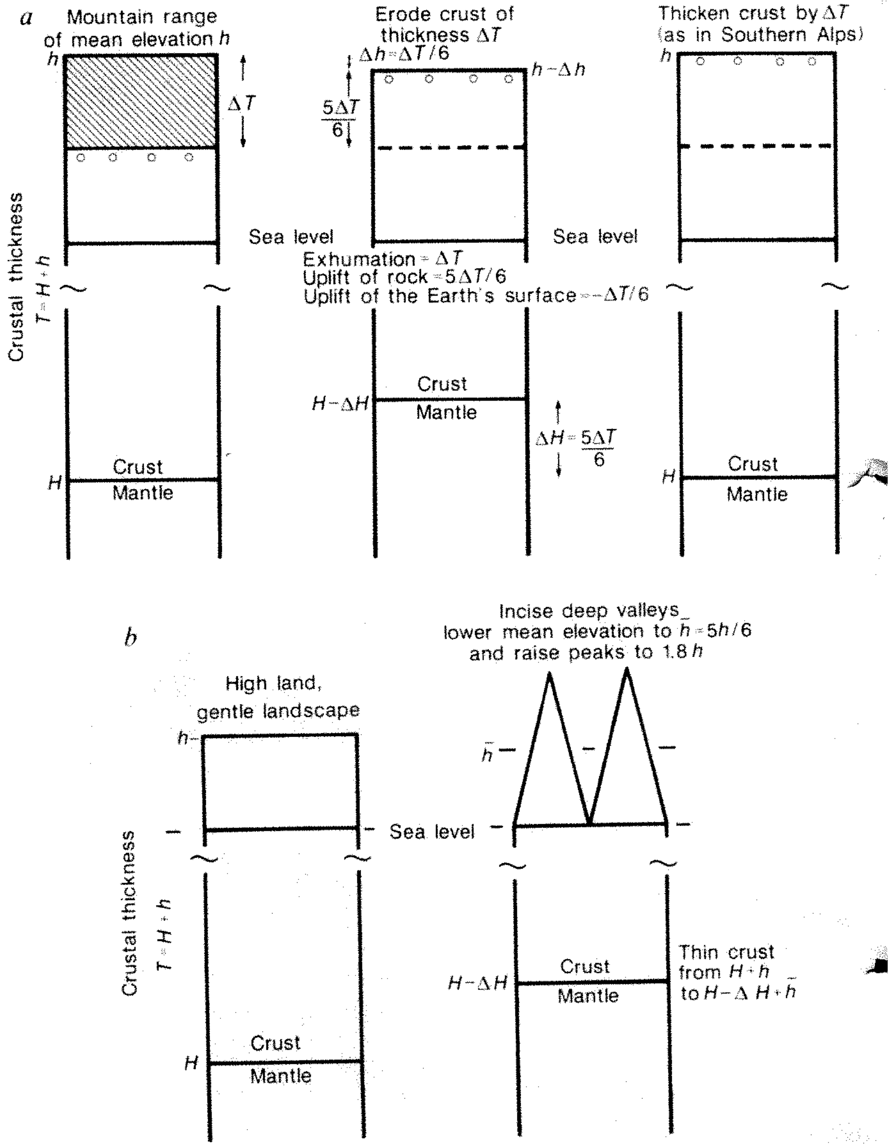
a
Mountain range of mean elevation h
h
ΔT
Crustal thickness T=H+h
Crust
Mantle
H
Erode crust of thickness ΔT
Δh=ΔT/6
h−Δh
5ΔT/6
Sea level
Exhumation=ΔT
Uplift of rock=5ΔT/6
Uplift of the Earth's surface=−ΔT/6
Crust
Mantle
H−ΔH
ΔH=5ΔT/6
Sea level
Thicken crust by ΔT (as in Southern Alps)
h
Crust
Mantle
H
b
High land, gentle landscape
h
Sea level
Crustal thickness T=H+h
Crust
Mantle
H
Incise deep valleys_ lower mean elevation to h̄=5h/6 and raise peaks to 1.8h
h̄
Crust
Mantle
H−ΔH
Thin crust from H+h to H−ΔH+h̄

FIG. 2 Simplified crustal sections showing the effects of erosion and crustal thickening on mean elevation, depth of the Moho (the crust/mantle boundary) and uplift of rock. *a*, Imagine a mountain range (left) of mean elevation h, depth to the Moho H and crustal thickness $T=H+h$, and allow a thickness of crust ΔT to be eroded. By isostasy, the mean elevation should decrease by $\Delta h \approx \Delta T/6$, and the underlying rock, including the Moho, should rise by $5\Delta T/6$ (centre). Hence, an exhumation of ΔT yields uplift of rock of $5\Delta T/6$, but a lowering of mean elevation of $\Delta T/6$. Only if, simultaneously, tectonically caused crustal thickening at depth replaces the thickness of crust, ΔT, lost by erosion, will the mean elevation and the depth to the Moho remain constant (right). This is what seems to be happening in the Southern Alps of New Zealand[50] (see text). *b*, Similarly, if a gentle highland of mean elevation h (left) were deeply incised by rivers that eroded valleys nearly to sea level (right), the mean elevation should drop slightly to about $5h/6$, the remaining rock and Moho would rise by an amount equal to h, and the highest peaks would be much higher than before. In this way, a climatically induced increase in erosion rate can cause exhumation of rocks, and hence the appearance of uplift, with no increase (in fact, with a decrease) in mean elevation.

changes) provide a direct measure of the uplift of the rocks concerned. But the heights of such terraces would equal the change in mean elevation of the terrain only if no material had been removed by erosion. The uplift of such terraces could be caused entirely by tectonic processes, if there were no erosion, or entirely by isostasy, if the uplift of these rocks were merely the isostatic response of the terrain to the removal of material by erosion.

Evidence used to infer recent uplift

We contend that many inferences of recent uplift of mountain ranges are based not on measures of uplift of the Earth's surface, but on estimates either of uplift of rocks or of exhumation[28]. Furthermore, some of the phenomena used as indicators of uplift could in fact be caused by climate change. An abrupt change in climate, as seems to have occurred at ~15 Myr[22], and as clearly occurred at ~2.5 Myr[23], is likely to affect the rate at which the landscape evolves. An increase in alpine glaciation will incise and denude high terrains, and changes in precipitation are likely to affect erosion rates. We suggest that a change towards a cooler and stormier climate could be responsible for the geomorphological evidence ('youthful landscapes') often used to infer recent uplift. Similarly, increased denudation and exhumation should yield an increase in the rate of coarse terrigenous sedimentation, a phenomenon also often attributed to uplift. Finally, global cooling has contributed to the change in mountain flora from warmth-loving species to those characteristic of cooler environments—palaeobotanical evidence that is commonly taken to indicate recent uplift of the fossil material relative to sea level.

Geomorphology. One of the observations commonly used to infer recent uplift is the sharp incision by streams and rivers into gentle surfaces mantled by late Cenozoic sediment. It is a basic tenet of geomorphology that the uplift of a surface with respect to the base level of the streams provides the streams with increased potential energy and steeper gradients, leading to rapid incision of the surface.

Such observations comprise the primary evidence for de Sitter's[30] postulation of 2,000 m of Pliocene uplift of the Pyrenees and Alps, and 1,000 m for the High Atlas mountains. From incised erosion surfaces at 1,000–2,000 m on the north flank of the Pyrenees and an extrapolation to 3,000 m near the crest of the range, de Sitter inferred 2,000 m of uplift since the beginning of the Pliocene epoch. Similarly, Trümpy[31] reported that the Pliocene Alps were 'not more than a hilly tract', whereas now deep valleys near sea level separate ridges with crests rising to 4,000 m. Because the present mean elevation of the Alps is only ~2,000 m[32], however, de Sitter's[30] inferred uplift of 2,000 m could simply be the isostatic response to the removal of material by the incision of the deep valleys (see Fig. 2*b*). It is easy to imagine that global cooling enabled alpine glaciers to grow and to excavate deep valleys. We do not suggest that the Alps and Pyrenees underwent no erosion for tens of millions of years before 2.5 Myr, but rather that a change in the processes of erosion and an increased erosion rate reshaped the gentle early Pliocene landscape into jagged relief.

Like those in the Alps and Pyrenees, the major structures of the Rocky Mountains of Colorado, Wyoming and Utah formed in late Cretaceous and early Cenozoic time. Partly because a high erosion surface has been deeply dissected in late Cenozoic time, much of the present elevation of the Rockies is nearly unanimously assigned to the Pliocene and/or Pleistocene epochs[33–36]. But, as "glacial erosion was the chief cause of the destruction of the Eocene surface in the higher parts of the mountains" (ref. 35, p. 244), here too, climate change should be considered as a contributor to accelerated erosion rates, and the appearance of recent uplift.

Sedimentation. Widespread rapid denudation manifests itself not only by the exhumation of rock, but also by an increased rate of deposition of sediment, near or far from the exhumed terrain. Thick deposits of late Cenozoic conglomerate surround most steep mountain ranges, and the large cobbles that comprise conglomerate imply transport along steep slopes. Thus, "tectonic activity is commonly held responsible for abrupt coarsening of alluvial fan sequences"[37]. Potter[38] used this argument to infer late Tertiary uplift of the southern Appalachians. Another of the arguments for recent uplift of the Rocky Mountains derives from the presence of thick late Cenozoic conglomerate near them[33,34,39]. Uplift of one block with respect to another can cause steep gradients, but, as Blackstone[33] noted for one basin in the Rockies, climate change also can affect the potential for streams to transport large cobbles. As Frostick and Reid[37] pointed out, infrequent but large storms can produce the abrupt coarsening of fanglomerate stratigraphy that is commonly attributed to tectonic processes.

Accelerated denudation is also revealed by sediment accumulation far from mountain ranges. Accumulation rates of pelagic sediment in the Atlantic, Indian and Pacific oceans in late Cenozoic time exceeded those for the rest of that era[40], with a four- to fivefold increase[41] in the rate of terrigenous deposition since 5 Myr. Like Donnelly[42], we suspect that the rapid increase in sediment accumulation throughout the world is a consequence of climate change.

The deposition rate since 2 Myr in the Gulf of Mexico, and in particular in the Mississippi delta, has been roughly four times that for the previous 60 Myr (see Fig. 3), and Hay *et al.*[43] argued that most of this material has come from the Rocky Mountains. Using constant quantitative relations among elevation differences, rates of sediment transport, base level to erosion, and an assumed history of eustatic sea-level change, they calculated a large late Cenozoic uplift of the Rocky Mountains from this thick Quaternary sediment. Because sediment transport rates also depend on climate[44–46], however, a different relationship between sediment transport and elevation for late Cenozoic and earlier times would yield a different, perhaps negligible, inferred Quaternary uplift of the Rockies.

The rapid denudation of the Southern Alps of New Zealand, where a wealth of observations indicate rapid uplift of rock with respect to sea level[47–49], illustrates the relationship of uplift of rock to both denudation and tectonics. Adams[50] showed that the regional erosion rate, determined both from suspended sediment in streams and from sediment accumulations around the island, is comparable to the rate of uplift of rock. Thus, the isostatic response to the removal of material must be a major cause of the present uplift of the marine terraces. To see this, remember that for each 1 km of material removed from the range, the isostatic response is a lowering of the mean elevation by only ~0.17 km (Fig. 2). Thus, although in the steady state that Adams[50] deduced, tectonic processes seem to maintain a supply of rock to be eroded (by thickening the crust), nevertheless, if tectonic processes stopped, five-sixths of the uplift of rock would still occur. Oblique convergence between the Pacific and Australian plates[51,52] has occurred in the region of the Southern Alps since 10–15 Myr[50], but virtually all of the sediment in the basins surrounding the Southern Alps seems to be younger than 2.5 ± 0.5 Myr. Thus, the recent increase in the rates of sedimentation and erosion correlates well with the onset of 'Pleistocene' glaciation, at least as dated in the Northern Hemisphere[23], but not with any resolvable change in plate motion[51,52].

Palaeobotany. The main cause of the enormous variation in vegetation over the Earth is the variation in climate. Thus, to use palaeobotany as a palaeobarometer, one must first use it as an indicator of palaeoclimate and then relate that climate to an inference of what the climate would have been in the same region at sea level.

One palaeobotanical approach used to infer palaeoclimates exploits qualitative relationships between assemblages of different taxa and the climates that characterize their present environments[53]. One assigns the fossil plants 'nearest living relatives', and then finds a present-day environment that includes

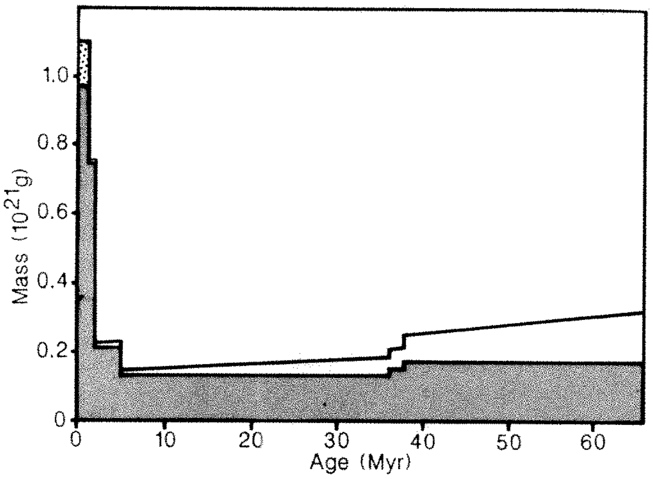

FIG. 3 Mass of Cenozoic sediment in the northwestern Gulf of Mexico and its drainage basin, between the Rocky Mountains and the Appalachians, as a function of the age of the sediment (from ref. 43). The blackened area shows existing sediment, minus an estimate by Hay *et al.* of the sediment transported by the Laurentide ice sheets from the Canadian shield in the past 1-2 Myr and deposited in this drainage basin. The line above shows their estimate of the sediment that was deposited and subsequently removed. Note that the huge increase in sedimentation at ~2 Myr correlates with the abrupt onset of widespread glaciation in the Northern Hemisphere[23].

as many of these relatives as possible. From the characteristics of that environment, one infers a likely palaeoclimate of the region[53]. The assignment of a nearest living relative is likely to be easiest for the most recent geological past; for Miocene and older times, a substantial fraction of the fossil species are extinct, and the conditions in which many of them lived must have been different from those of their descendants. For example, Axelrod[54] found that most of the nearest living relatives of fossil taxa from the Eocene Copper River formation in northeastern Nevada grow in either of two types of forest: coastal redwood forests, where elevations range from sea level to ~150 m, and fir-hemlock forests in the Sierra Nevada, at elevations of 1,400-2,400 m. Clearly, assigning a palaeo-elevation (or palaeoclimate) to such a fossil assemblage from present-day equivalents requires an element of subjectivity that makes the method qualitative at best[55].

Moreover, sometimes the taxonomic classification is based on parts of a plant, such as pollen, that can be relatively insensitive to climate change. Fossil pollen may have fallen from trees whose leaves and wood have since evolved to be adapted to a different climate, while the pollen remains similar. As an extreme analogy, imagine finding late Quaternary tusks in the Siberian Arctic, matching them to the nearest living relative, the elephant, and concluding that because elephants now live in hot, dry savannas at low latitudes, the present North Pole also lay at tropical latitudes in the late Quaternary era. Tusks are not adaptations to climate.

A second palaeobotanical approach to palaeoclimate ignores, insofar as is possible, the taxonomy of the fossil plants and simply uses what is known of how plants adapt to different climates. Using trees from eastern Asia, Wolfe[56] showed that the percentage of species having leaves with smooth, continuous margins ('whole margins') varies linearly with mean annual temperature, with a scatter of only 2 °C. The application of physiognomic characteristics to palaeoclimates is not without limitations[56], but climate changes inferred from the physiognomy of fossil plants have been corroborated. From leaf shapes, Wolfe[15,19,20] deduced an abrupt drop in temperature of several degrees in only a couple of million years near the Eocene/Oligocene boundary. Oxygen isotopes from the sub-Antarctic region near New Zealand later corroborated this rapid decrease in both surface and bottom water temperatures[16,21]. Using nearest living relatives, however, Axelrod and Bailey[57] inferred only a gradual change.

Virtually all attempts to use palaeobotany to infer palaeo-elevations have relied on taxonomy, not physiognomy. Moreover, many studies, such as those reporting recent uplift of the Tibetan plateau or the Himalayas[58-61], have ignored global climate change. Given that most of the Earth cooled during the late Cenozoic era[15-18], it is not surprising that palaeobotanical observations make it seem that mountain ranges have risen recently. For an average lapse rate of $\sim 6\,^{\circ}\mathrm{C\,km^{-1}}$, a secular change in temperature of 6-9 °C since 5-15 Myr[16-18] could yield an erroneous inference of 1,000-1,500 m of uplift. Moreover, because environmental lapse rates tend to be lower than free-air lapse rates[62], such a change in mean annual temperature could lead to a larger error in inferred uplift. Thus, although both the Himalayas and Tibet rose in Cenozoic time, the palaeoclimatic inferences of an accelerating rise throughout Cenozoic time[58-61] (see Fig. 4) are likely to be exaggerated, if not simply false[28].

The Rocky Mountains provide another example of exaggerated, palaeobotanically determined, late Cenozoic uplift. MacGinitie[63] noted that many of the species from the early Oligocene Florissant flora in central Colorado could not be matched with present-day equivalents from any single, well defined forest; nevertheless, he inferred a relatively warm mean annual temperature of 18 °C and a palaeo-elevation of less than 915 m. The evidence for warm climates where early Eocene (Laramide) deformation had created mountain ranges is a common argument for low palaeo-elevations[39,64,65], and the palaeo-elevation of Florissant has provided a base level for estimates of subsequent uplift of the whole of the Rockies and the Great Plains[65]. Recall, however, that the late Eocene/early Oligocene climate was very warm—warmer than it has been since then[15-18].

Using the physiognomy of the fossil leaves of the Florissant assemblage, Meyer[66] inferred a mean annual temperature of 14 °C, not greatly different from MacGinitie's 18 °C. By comparing this with the likely warm early Oligocene sea-level temperature at the same latitude, however, and using an appropriate lapse rate and a 200-m difference between early Oligocene and present sea level, Meyer[66] inferred a palaeo-elevation of 2,450 m. Even if a smaller sea-level difference were used, this would be indistinguishable from the present elevation of 2,600 m. He also inferred a high palaeo-elevation of 2,800-3,500 m for the nearby Marshall Pass flora of roughly the same age, presently at 2,835-3,080 m. Moreover, from the physiognomic characteristics of the late Oligocene Creede flora, at 2,680 m in central Colorado, Wolfe and Schorn[67] inferred a mean annual temperature of 0-2.5 °C, which is virtually identical to the present mean annual temperature of 1.9 °C. Ironically, because Wolfe and Schorn[67] were prejudiced by the prevailing opinion that the Rockies rose only in late Cenozoic time, they suggested that the Creede flora were deposited in a basin where cold air could be trapped. Thus, Meyer's[66] and Wolfe and Schorn's[67] analyses, based on physiognomic characteristics of fossil plants and on differences in present and past climates, show that differences between past and present taxa should not be taken as evidence of low elevations in the western United States in mid-Cenozoic time and of a subsequent recent increase in the mean elevation of the Rockies.

Climate change and the appearance of uplift

The arguments above are intended to show that most estimates of late Cenozoic uplift of mountain ranges are exaggerated, at least if changes in mean elevation define uplift, and to suggest that much of the evidence used to infer recent uplift is a consequence of climate change. Most palaeobotanical inferences of recent uplift of the Earth's surface ignore global cooling, which biases these estimates upward. Inferences of changes in mean elevation from elevated geomorphic surfaces, increased denudation or increased sedimentation overlook the parts played by erosion, which depends on climate[44-46], and by isostatic rebound. Isostatic compensation of material removed by erosion will elevate the remaining terrain, but the uplift of ridges and peaks with respect to sea level does not necessarily signify an increase in mean elevation. Our attribution of the rapid erosion of mountain ranges in late Cenozoic time to climate change must remain a hypothesis, given that climate change can be very different in different places[13], that different types of local climate change can increase erosion rates[44-46], and that it is difficult to distinguish a local climate change due to global processes from one due to local uplift. Nevertheless, the same difficulties face the geologist trying to show that the recent rapid incision of a major mountain range is due to uplift, instead of global climate change.

The correlations of global climate change with the phenomena used to infer uplift offer some support for the idea that climate change is responsible for these phenomena. The physiognomy of Oligocene fossil plants from the Rocky Mountains[63,66,67] is consistent with the climates that would be expected at present elevations, if known global climate changes[15-18] are allowed for, but with lower elevations if that global cooling is ignored. Moreover, much of the deep incision of the erosion surface across the Rocky Mountains was due to glaciation[35]. In fact, most major mountain ranges thought to have risen in late Cenozoic time have been glaciated, and glaciation increased greatly as the Earth became cooler. The onset of rapid sedimentation in New Zealand at ~2.5 Myr[50], due to rapid erosion of the Southern Alps, correlates with the onset of late Cenozoic cooling and glaciation[23], but not with any obvious change in tectonics[51,52]. Finally, late Cenozoic uplift has been inferred for mountain ranges throughout the world, yet globally synchronous changes in plate motions, if they have occurred, have been small. Climate change, on the other hand, has been a global phenomenon.

Although the physical mechanisms by which global climate change might affect erosion rates could be different in different regions, several plausible processes are likely to have enhanced erosion. At relatively high altitudes or high latitudes, alpine glaciation is likely to be the dominant process of erosion, and global cooling surely enhanced glaciation. In the absence of glaciation, the principal "agents of erosion and transportation are the impact of raindrops and the surface flow of water"[46], but variations in precipitation can affect erosion in such extreme ways that one cannot assume that more precipitation would lead to more erosion, or vice versa. In some climates, increased precipitation can increase erosion, but in others, a decrease in precipitation can increase erosion, by reducing the amount of protective vegetation[68]. Whether global cooling led to increased or decreased precipitation is difficult to decide (see below) and likely to vary regionally.

The atmosphere is a thermal engine, which absorbs the Sun's energy in the tropics and transports it upwards and polewards as both sensible heat and latent heat in water vapour. Precipitation in middle latitudes is strongly affected by the ability of the atmosphere to transport humid air from the tropics to cooler higher latitudes. This transport is accomplished primarily by large-scale eddies generated by baroclinic instability[27], the same instability that makes the climate stormy. Two aspects of the temperature field affect the rate of transport: the strong temperature dependence of the saturated mixing ratio of vapour and dry air, and the latitudinal temperature gradient. The mixing ratio is proportional to $\exp(-L/RT) = \exp(-5{,}400/T)$, where L is the latent heat of vaporization, R is the dry-air gas constant, and T is temperature in degrees Kelvin[27]. The strong dependence of the mixing ratio on temperature makes cooler air much drier than warm air, implying that global cooling would lead to drier climates and less precipitation. The rate of transport, however, is also proportional to the square of the latitudinal temperature gradient. Thus, the inferred change from ~0.25 to 0.5 K per degree of latitude since early Cenozoic time[15] would yield a fourfold increase in latitudinal transport of moisture, but a decrease in mid-latitude temperature of 10 K would yield only a 1.8-fold decrease in water vapour transported. This kind of calculation, which ignores the reduced evaporation at middle latitudes at lower temperatures, is too imprecise to allow a definitive statement about changes in precipitation during Cenozoic time, but it emphasizes that global cooling would not necessarily reduce precipitation. More importantly, the increased latitudinal temperature gradient should have enhanced baroclinic instability, making the climate stormier. Annual erosion rates in many areas are dominated by a few storms[44,46]; thus, even if global cooling did lead to a drying, as some general circulation model runs suggest[69], the increased storminess could have increased net erosion.

Climate change and uplift: chicken and egg?

The temporal correlation of late Cenozoic glaciation with phenomena commonly ascribed to recent uplift is inescapable, but assigning the ultimate cause of late Cenozoic climate change to late Cenozoic uplift arising from tectonic processes is a hypothesis that we reject. Instead, we have suggested here that

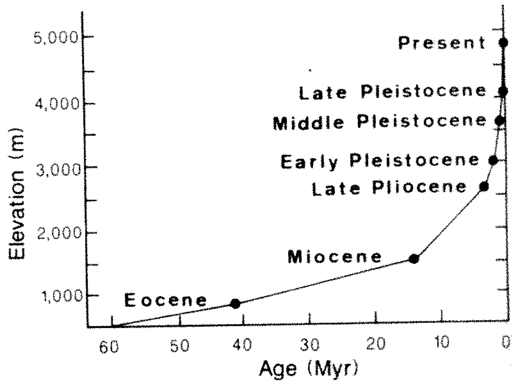

FIG. 4 Inferred history of uplift of the Tibetan Plateau and the Himalayas, based on palaeobotanical data largely from the north slope of the Himalayas. (Redrawn from ref. 60.) Fossil species whose nearest living relatives grow at relatively low elevations and in relatively warm climates were used to infer lower past elevations, but no account was taken of global cooling, as illustrated in Fig. 1. We show this as an example of inferred uplift, but we doubt that it is precise, or that it applies to all of the Tibetan plateau.

the phenomena commonly used to infer recent uplift are a consequence of climate change.

This is not to dismiss entirely the processes linking elevation and climate described by Birchfield, Kutzbach, Raymo, Ruddiman and Weertman[9-14]; indeed, there is evidence for their operation in a longer-term correlation (if only crude) of climate change with the growth of high terrain in the past 50 Myr[10]. There was no Tibetan plateau in late Cretaceous time, when marine limestones were being deposited in the region[70]. Moreover, because the present tectonic activity in Asia seems to be due to the collision of India withEurasia in Cenozoic time, the high mean elevation of much of the rest of eastern Asia was also probably acquired during Cenozoic time. Thus, the gradual cooling over the past 50 Myr might be the consequence of the tectonic processes that built Tibet, the Himalayas and other high terrains in Asia.

It follows that changes in mean elevation, climate change and the phenomena commonly used to infer uplift are coupled to one another. Although the physical mechanisms linking climate change to uplift should apply most strongly to regions where mean elevation has increased, they could also operate where crests of ranges have risen in response to erosion and isostatic rebound. This raises the possibility that climate change, weathering, erosion and isostatic rebound might interact in a system of positive feedback. Suppose increased weathering and erosion could enhance a negative 'greenhouse effect'[14]; then, if climate change accelerated erosion and weathering, the associated withdrawal of carbon dioxide from the atmosphere might cause further global cooling. Increased rates of erosion and isostatic rebound would alter the distribution of elevations by making the crests of ranges higher than before. The prolonged duration of winter and snow cover in such higher areas might lead to further reductions in temperature[11]. In addition, mountain ranges with high crests and deep valleys will perturb atmospheric circulation more strongly than those with the same mean elevation but lower crests. Thus, if the rise of crests of mountain ranges were to perturb the climate in such a way as to enhance erosion, isostatic rebound would cause a further rise, and further perturbations to the climate. By such feedback mechanisms, the gradual regional uplift of the Earth's surface in Asia, due to Cenozoic tectonics, might have led to an accelerating climate change and the illusion of accelerated uplift of mountain ranges throughout the world. □

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The protein encoded by the *Arabidopsis* homeotic gene *agamous* resembles transcription factors

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Mutations in the homeotic gene *agamous* of the plant *Arabidopsis* cause the transformation of the floral sex organs. Cloning and sequence analysis of *agamous* suggest that it encodes a protein with a high degree of sequence similarity to the DNA-binding region of transcription factors from yeast and humans and to the product of a homeotic gene from *Antirrhinum*. The *agamous* gene therefore probably encodes a transcription factor that regulates genes determining stamen and carpel development in wild-type flowers.

ARABIDOPSIS THALIANA is widely used for molecular and genetic studies of many developmental processes in plants[1,2]. We have focused on the molecular basis of pattern formation in developing *Arabidopsis* flowers. Flower development remains one of the most complex processes unique to plants, and little is known about the molecular mechanisms involved. Flowers develop from a few undifferentiated cells into a structure that has several types of organs occupying precisely defined positions. *Arabidopsis* has typical mustard flowers (Fig. 1*a*) consisting of four concentric whorls of organs[3]. The first or outermost whorl consists of four green sepals. Interior to and alternating with the sepals are four white petals which make up the second whorl. The third whorl consists of six stamens, four long and two short, each of which has a filament capped by a pollen-bearing anther. The fourth and innermost whorl consists of two fused carpels capped by a short style and stigmatic papillae.

Many mutations affecting floral morphogenesis have been identified in *Arabidopsis*. Mutations in at least four genes result in homeotic transformations of floral organs[1,3-7]. In general, these homeotic mutations affect the development of adjacent whorls of floral organs. For example, the *ap2-1* mutation results in the conversion of the sepals into leaf-like organs, and in the conversion of petals into stamen-like organs[3]. Mutations in the *AP3* (*ap3-1*) or *PI*(*pi-2*) genes result in the conversion of petals into sepals and stamens into carpels. Mutations in the *agamous* (*AG*) gene result in the overall phenotype of a flower within a flower and the absence of stamens and carpels. The six stamens have been replaced in *ag* mutant flowers by six petals, and the carpels have been replaced by a new flower. Thus, *ag* flowers consist of ten petals (four normal and six in the place where stamens are normally found) inside of the four sepals, and inside the petals are again four sepals and ten petals (Fig. 1*c*). This pattern repeats itself and the resulting flower can consist of more than 70 organs. Other species of plants with similar double flower phenotypes were recognized as long ago as 2,000 years[8]. The first published report of *Arabidopsis* flowers with an *ag* mutant phenotype was more than a century ago[9], and another *Arabidopsis* mutant having similar flowers has been described by Conrad[10]. The extensively characterized[3] mutant allele, *ag-1*, was isolated after ethylmethane sulphonate (EMS) mutagenesis and was first described by Koornneef *et al.*[11]. The *AG* locus has been mapped to chromosome 4 (ref. 11).

Here we describe the molecular cloning and characterization of the *AG* gene, which was facilitated by a T-DNA insertion mutation[12]. The deduced AG protein product is similar to transcription factors from humans (SRF) and yeast (MCM1, ARG80), and to the product, DEF A, of a recently isolated homeotic gene from the snapdragon *Antirrhinum majus*.

Insertion mutant of *agamous*

Recently, a method has been developed to tag genes by insertion of the T-DNA from *Agrobacterium tumefaciens*[13]. One of the

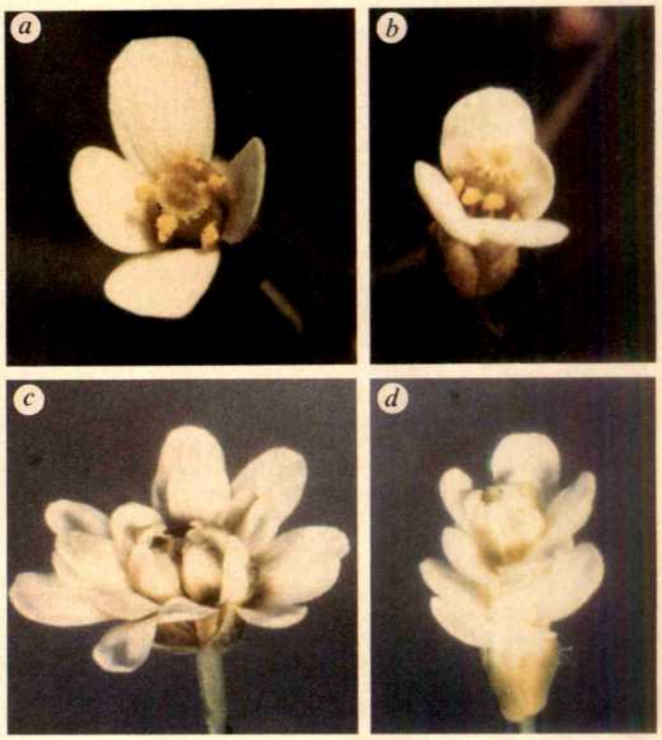

FIG. 1 Photographs of *Arabidopsis* flowers. *a*, Wild-type; *b*, *ag-2* plants transformed with pCIT540; *c*, *ag-1*; *d*, *ag-2*. Complementation of the *ag-2* mutation was tested by introducing the cosmid pCIT540 into the *Agrobacterium* strain ASE (ref. 26). This strain was used to infect leaf pieces of homozygous *ag-2* mutant plants by standard methods[27]. A regenerated plant that displayed wild-type flower morphology is shown here.

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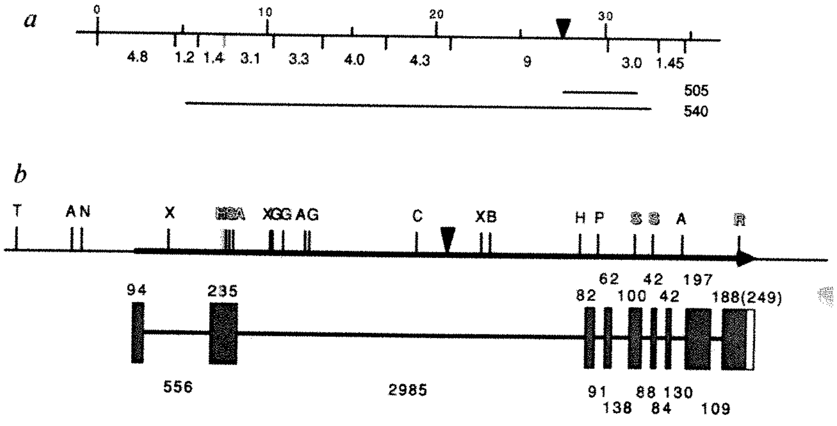
mutants isolated by this approach (Fig. 1*d*) displays a phenotype that closely resembles that of *ag-1* mutant plants (Fig. 1*c*). Like *ag-1*, this insertional mutant lacks stamens and carpels and has the overall phenotype of a flower within a flower. The only noticeable difference between the insertional mutant and *ag-1* mutant plants is that the pedicels of inner flowers in the former are elongated, resulting in a greater separation of internal flowers. The elongated pedicel is not due to the insertional mutation, but is the result of different genetic backgrounds (M.F.Y., H.M., J.L.B., G.N.D., K.A.F. and E.M.M., unpublished results). To demonstrate whether the insertional mutation is an allele of *AG*, plants heterozygous for *ag-1* were crossed to plants heterozygous for the insertional mutation (homozygous mutant plants could not be used because they are sterile). As the *ag-1* and insertional mutants are both recessive to wild-type, and as a quarter of the resulting F1 progeny produced *ag* mutant flowers, this confirms that the two mutations are allelic. We have named the insertional allele *ag-2*.

Genetic analyses indicate that the kanamycin resistance marker encoded by the T-DNA co-segregates with the mutant flower phenotype (data not shown). This, together with the demonstration that the insertion mutation is allelic to *ag-1*, indicates that the T-DNA can be used as a molecular probe to isolate the *AG* gene. Plasmid rescue was used to isolate sequences flanking the insertion site and one of the resulting clones, pCIT505, contained plant sequences and was analysed further (Fig. 2*a*). To determine whether these sequences were derived from the *AG* genomic region, the genetic map position of the



c

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     CTAAATGTACTGAAAAGAAACACCAGTTTAATTAATTATACTTTCCTCACATATAACTATCAACCAACTACAAAACTTTTGTC
     AATTCTCAAAATCAACTTTCACCACATAATTATCTAACATGTGTATGTTCCAAAACCAGTTTAAATGAATTACTTTTCAGAAA
     ATACATGTATATTAACTCTATCTAATAAAGAAGAAACACATACTTATCTCATAGATTCCATTCATAAAACTATGCTTTAGTGA
     GTAAGAAAACCAGTAATCAAACACAAATTGACAAGACACTATATGGATGTAAAAAGTGGGAAAATATGGTCATAAATAGTAC
     AGAAAATTAAAAAGAAAAAATAATATTCCTTTATAAATGTATATAGCCATCTCTTCACCAGCACAACCTTACCTTCCATTTTC

   1 CAT TTT CTG CAA CTT CTC CAA ATC TCA TAC TTT CCA GAA AAT CAT TTT CCC AAG
   1 His Phe Leu Gln Leu Leu Gln Ile Ser Tyr Phe Pro Glu Asn His Phe Pro Lys
                                                          ▼
  55 AAA AAT AAA ACT TTC CCC TTT GTT CTT CTC CCC CCA ACA GCA ATC ACG GCG TAC
  19 Lys Asn Lys Thr Phe Pro Phe Val Leu Leu Pro Pro Thr Ala Ile Thr Ala Tyr
          *
 109 CAA TCG GAG CTA GGA GGA GAT TCC TCT CCC TTG AGG AAA TCT GGG AGA GGA AAG
  37 Gln Ser Glu Leu Gly Gly Asp Ser Ser Pro Leu Arg Lys Ser Gly Arg Gly Lys

 163 ATC GAA ATC AAA CGG ATC GAG AAC ACA ACG AAT CGT CAA GTC ACT TTT TGC AAA
  55 Ile Glu Ile Lys Arg Ile Glu Asn Thr Thr Asn Arg Gln Val Thr Phe Cys Lys

 217 CGT AGA AAT GGT TTG CTC AAG AAA GCT TAC GAG CTC TCT GTT CTC TGT GAT GCT
  73 Arg Arg Asn Gly Leu Leu Lys Lys Ala Tyr Glu Leu Ser Val Leu Cys Asp Ala

 271 GAA GTC GCA CTC ATC GTT TTC TCT AGC CGT GGT CGT CTC TAT GAG TAC TCT AAC
  91 Glu Val Ala Leu Ile Val Phe Ser Ser Arg Gly Arg Leu Tyr Glu Tyr Ser Asn
          ▼
 325 AAC AGT GTA AAA GGT ACT ATT GAG AGG TAC AAG AAG GCA ATA TCG GAC AAT TCT
 109 Asn Ser Val Lys Gly Thr Ile Glu Arg Tyr Lys Lys Ala Ile Ser Asp Asn Ser
                                                ▼
 379 AAC ACC GGA TCG GTG GCA GAA ATT AAT GCA CAG TAT TAT CAA CAA GAA TCA GCC
 127 Asn Thr Gly Ser Val Ala Glu Ile Asn Ala Gln Tyr Tyr Gln Gln Glu Ser Ala
                                                          ▼
 433 AAA TTG CGT CAA CAA ATT ATC AGC ATA CAA AAC TCC AAC AGG CAA TTG ATG GGT
 145 Lys Leu Arg Gln Gln Ile Ile Ser Ile Gln Asn Ser Asn Arg Gln Leu Met Gly

 487 GAG ACG ATA GGG TCA ATG TCT CCC AAA GAG CTC AGG AAC TTG GAA GGC AGA TTA
 163 Glu Thr Ile Gly Ser Met Ser Pro Lys Glu Leu Arg Asn Leu Glu Gly Arg Leu
                                                ▼
 541 GAG AGA AGT ATT ACC CGA ATC CGA TCC AAG AAG AAT GAG CTC TTA TTT TCT GAA
 181 Glu Arg Ser Ile Thr Arg Ile Arg Ser Lys Lys Asn Glu Leu Leu Phe Ser Glu
                                ▼
 594 ATC GAC TAC ATG CAG AAA AGA GAA GTT GAT TTG CAT AAC GAT AAC CAG ATT CTT
 199 Ile Asp Tyr Met Gln Lys Arg Glu Val Asp Leu His Asn Asp Asn Gln Ile Leu
                ▼
 649 CGT GCA AAG ATA GCT GAA AAT GAG AGG AAC AAT CCG AGT ATA AGT CTA ATG CCA
 217 Arg Ala Lys Ile Ala Glu Asn Glu Arg Asn Asn Pro Ser Ile Ser Leu Met Pro

 703 GGA GGA TCT AAC TAC GAG CAG CTT ATG CCA CCA CCT CAA ACG CAA TCT CAA CCG
 235 Gly Gly Ser Asn Tyr Glu Gln Leu Met Pro Pro Pro Gln Thr Gln Ser Gln Pro

 757 TTT GAT TCA CGG AAT TAT TTC CAA GTC GCG GCA TTG CAA CCT AAC AAT CAC CAT
 253 Phe Asp Ser Arg Asn Tyr Phe Gln Val Ala Ala Leu Gln Pro Asn Asn His His
                                                              ▼
 811 TAC TCA TCC GCC GGT CGC CAA GAC CAA ACC GCT CTC CAG TTA GTG TAA
 271 Tyr Ser Ser Ala Gly Arg Gln Asp Gln Thr Ala Leu Gln Leu Val *

 860 TATTGGCTGAAGGAAATGGCCTGGAGTGAATAAAAACCAGAATTGGGTTGAGCAAGCAATATAAAGCTAAT
 931 GCATGTTATATATATATTTATCCCATGAATGTTGTATCAGTGAATTCTTATGCTTATGTTGATGTGAAATT
1002 AATATCTTAAAGACATGTCATTAATGTGCTTAATTTGCTTCA
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FIG. 2 *a*, Map of the *agamous* region. *Eco*RI restriction sites are shown for a 35-kb region from the Columbia ecotype flanking the *AG* gene. Size of fragments is in kb. ▼, site of T-DNA insertion in the *ag-2* mutant. Below the restriction map, horizontal lines indicate the region contained in the plasmid-rescued clone pCIT505, and the cosmid pCIT540. *b*, Structure and restriction map of *AG* gene. The diagram below the restriction map is of introns (lines) and exons (shaded boxes); size in nucleotides is given. One cDNA extended an additional 61 nucleotides (open box). Restriction sites include: A, *Sca*I; B, *Bam*HI; C, *Nco*I; H, *Hin*dIII; N, *Nde*I; S, *Sac*I; R, *Eco*RI; T, *Pst*I; X, *Xba*I. Restriction sites in outline indicate sites in exon sequences. ▼, site of the 35-kb T-DNA insertion in *ag-2* allele. Arrow, direction of transcription. *c*, Nucleotide sequence of *AG* cDNAs and the deduced amino-acid sequence for the open reading frame. Nucleotide 1 corresponds to the first nucleotide in the longest isolated cDNA and nucleotide 1,043 is the site of polyadenylation. The region preceding nucleotide 1 was derived from genomic sequence. ▼, position of intron sequences as deduced from a comparison of cDNA and genomic sequence data. *, position of the translational initiation codon for each of the *B. napus* genes.

METHODS. Cosmids were isolated from genomic libraries of nuclear DNA prepared from the Columbia and Landsberg *erecta* ecotypes of *Arabidopsis*. This DNA was partially digested with *Sau*3A, size fractionated by sucrose gradient centrifugation, ligated to the vector pCIT30 and packaged. Plasmid pCIT30 was derived from the plasmid pMON200 (ref. 26) by replacing the kanamycin resistance gene with a fragment that encodes hygromycin resistance. Plasmid rescue was achieved by digesting DNA from homozygous *ag-2* mutant plants with *Sal*I, followed by ligation and transformation into *Escherichia coli*. The digest with *Sal*I produces a fragment containing an *E. coli* origin of replication and the ampicillin resistance (Amp^r) gene encoded by the T-DNA, as well as plant sequences flanking the insertion[28]. Plasmid pCIT505 was placed on the RFLP map of Chang *et al.*[14] using an *Eco*RI RFLP and found to reside at about the same position as *ag-1*. To map directly pCIT505 relative to *ag-1* recombinants derived from a cross between Landsberg *erecta* (Ler) and Niederzenz (Nd-O) ecotypes that included the *ag-1* mutation were analysed. Plants heterozygous for the *ag-1* mutation (*ag-1* (Ler)/+(Ler)) were crossed to plants that were wild-type for *AG* in the Nd-O background (+(Nd-O)/+(Nd-O)). The resulting F1 progeny that were heterozygous for the *ag-1* mutation (*ag-1* (Ler)/+(Nd-O)) were allowed to self-fertilize and DNA was isolated from the F2 plants that displayed the *ag* phenotype (*ag-1*/*ag-1*). DNA blots were prepared and hybridized with probes revealing *Eco*RI and *Bgl*II RFLPs. The cDNA library was prepared from 2 μg poly(A)$^+$RNA isolated from immature floral buds[3,20] before stage 12 using the Stratagene kit. After size-selection of the cDNA (>400 bp) *Eco*RI linkers were added before ligating to the vector λgt10 and packaging using Gigapack gold (Stratagene). Many cDNA clones (3×10^6) were obtained, a portion of which were screened with a probe derived from the region flanking the T-DNA insertion site in *ag-2* mutant plants. Twenty seven of the longest independently isolated cDNAs were subcloned and fully or partially sequenced. DNA sequence analysis of one of these clones, pCIT559, demonstrated that it contained the fourth (138 base) intron. All of the other cDNAs seemed to represent the same fully processed RNA. Sequence analysis was performed on double-stranded plasmids using the USB Sequenase Kit. Both strands of cDNAs and the corresponding genomic region were sequenced.

sequences in pCIT505 was determined by restriction fragment length polymorphism (RFLP) analysis[14]. No crossovers were observed between a pCIT505-linked polymorphism (Fig. 2*a*) and the *ag-1* mutation in 118 meiotic products scored, indicating that this cloned segment maps less than two centimorgans from the *AG* gene. Taken together, these results suggest that the T-DNA is inserted in or near the *AG* gene and that we have recovered DNA sequences from this region. The pCIT505 plasmid was then used as a probe to screen a cosmid library of wild-type *Arabidopsis* sequences and several clones were isolated and characterized. One of these cosmids, pCIT540 (Fig. 2*a*), when introduced into the genome of homozygous *ag-2* mutant plants, complemented the mutation and the resulting flowers appeared to be of wild type, with stamens and carpels (Fig. 1*b*). As the T-DNA insertion has been stably maintained through eight generations, and the introduced sequences restore a wild-type flower phenotype to this stable strain, it seems that this region indeed contains the wild-type *AG* gene.

Nucleotide sequence

Restriction fragments spanning the T-DNA insertion site were used to probe a complementary DNA library prepared from RNA isolated from wild-type *Arabidopsis* flowers. Only one class of cDNA clones spanned the T-DNA insertion site. The nucleotide sequence of several overlapping cDNA clones and the corresponding genomic region was determined (Fig. 2*c*). These results indicate that this gene contains at least nine exons ranging in size from 42 to 235 bases, and eight introns ranging in size from 84 to 2,985 bases (Fig. 2*b*). Sequence analysis of the T-DNA-plant junction fragment indicates that the foreign DNA in the *ag-2* allele is inserted in the 2,985-base intron (Fig. 2*b*).

To confirm that these sequences represent the *AG* gene, genomic fragments were isolated and sequenced from the EMS-generated mutant *ag-1*. The *ag-1* mutation was induced in the same genetic background, Landsberg *erecta*, from which we have isolated and characterized the wild-type sequences. This analysis revealed a single nucleotide change that is presumably responsible for the *ag-1* mutant phenotype. This nucleotide substitution changes the AG dinucleotide to AA at the acceptor site of the fourth (138-base) intron shown in Fig. 2*b*.

The deduced protein product contains at least 285 amino-acid residues, although from analysis of the sequences it seems that none of the cDNA clones are of full length. As the deduced open reading frame continues to the 5′ end of the longest cDNA, and a translational initiation codon has not been identified, the full-length AG protein could be slightly longer. But many attempts with primer extension, anchored polymerase chain reaction and the screening of two different cDNA libraries have been unsuccessful at obtaining longer clones. Nearly all of the isolated cDNAs end within a region of a few nucleotides, suggesting that the RNA may have a secondary structure that prevents extension beyond this region. As the longest cDNAs are of 1,043 base pairs (bp) and the polyadenylated messenger RNA is close to 1.1 kilobases (kb) in length (see below), these cDNAs are of nearly full length. We have recently isolated the two presumed *AG* homologues from the allotetraploid mustard *Brassica napus* and each of these genes contains a translational initiation codon corresponding to the TCG codon at nucleotides 112-114 of the *AG* cDNA sequence (M.F.Y., H.M. and E.M.M., unpublished results). This indicates that all the evolutionarily conserved sequences of the *Arabidopsis* gene have been identified.

The deduced *AG* amino-acid sequence was used to search the database of protein sequences using the program of Lipman and Pearson[15]. The AG protein shares significant sequence similarity to a class of transcription factors from humans[16] and yeast[17,18], and to the product of a recently isolated *Antirrhinum* homeotic gene[19] (see Fig. 3).

```
                      10        20        30        40        50
                       |         |         |         |         |
AG         52 RGKIEIKRIENTTNRQVTFCKRRNGLLKKAYELSVLCDAEVALIVFSSRGRLYEYS
DEF A       3 RGKIQIKRIENQTNRQVTYSKRRNGLFKKAHELSVLCDAKVSIIMISSTQKLHEYI
SRF       144 RVKIKMEFIDNKLRRYTTFSKRKTGIMKKAYELSTLTGTQVLLLVASETGHVYTFA
MCM1       18 RRKIEIKFIENKTRRHVTFFKRKHGIMKKAFELSVLTGTQVLLLVVSETGLVYTFS
ARG80      80 RRKQPIRYIENKTRRHVTFSKRRHGIMKKAYELSVLTGANILLLILANSGLVYTFT

AG/DEF A      RGKI IKRIEN TNRQVT  KRRNGL KKA ELSVLCDA V  IV SS   L EY    41/56
AG/MCM1       R KIEIK IEN T R VTF KR  G  KKA ELSVL    V L V S  G  Y  S   32/56
AG/SRF        R KI    I N   R  TF KR  G  KKAYELS L    V L V S  G  Y      25/56
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FIG. 3 Amino-acid sequence comparison (single-letter code). An alignment is shown for the deduced amino-acid sequences for the gene products from *Arabidopsis thaliana* (AG), *Antirrhinum majus* (DEF A), humans (SRF) and yeast (MCM1, ARG80). Identical residues between AG and each of DEF A, MCM1 and SRF are shown below, as are the fractions of identical amino acids shared by these proteins. Numbers to the left of each sequence indicate the position of the first amino acid shown for each protein.

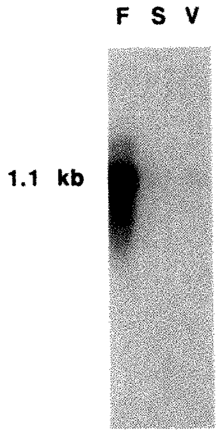

FIG. 4 RNA blot. An autoradiogram is shown for an RNA blot hybridized with ^{32}P-labelled *AG* cDNA. RNA was from immature floral buds (lane F), floral stems (lane S) and from vegetative tissue (lane V).
METHODS. Poly(A)$^+$RNA was isolated from *Arabidopsis thaliana* Landsberg (*er*) as previously described[29] and 2 μg each RNA type was size-fractionated on a 1.5% formaldehyde agarose gel, transferred to Hybond N nylon membrane (Amersham), and hybridized with a radiolabelled gel-isolated DNA fragment from the *AG* cDNA, pCIT565. Hybridization was in 50% formamide, 5 × SSC buffer, 10 × Denhardt's buffer, 100 μg ml^{-1} single-stranded salmon sperm DNA and 10% Dextran sulphate. The filter was washed three times in 2 × SSC, 1% SDS at room temperature and then four times in 0.2 × SSC, 1% SDS at 58 °C. The flower RNA was prepared from a collection of young floral buds before stage 12 (before the flowers open)[3,20]. Vegetative RNA was obtained by growing plants in a liquid culture for ~18 days before collection; the resulting tissue consisted of roots, stems and leaves. Floral stem RNA was prepared from floral stems with cauline leaves. The vegetative and floral stem RNA preparations also contain a small amount of floral RNA as these tissues contain immature stage-3 flowers[20]. To prove that RNA was present in the floral stem and vegetative lanes, this RNA blot was also hybridized with a cDNA specific for the *Arabidopsis* G-protein gene *GPA1* (ref. 30); this clone hybridized more strongly to the stem and vegetative RNA (lanes S and V) than to flower RNA.

RNA analysis

To study the pattern of *AG* gene expression, poly(A)$^+$ RNA was isolated from several different tissue types and hybridized with an *AG* cDNA. An RNA from flowers (length ~1.1 kb) hybridized with the *AG* cDNA probe (Fig. 4, lane F). After a much longer exposure, a similarly sized transcript was detected in RNA from inflorescence stems and whole plants before the appearance of the primary inflorescence stem (Fig. 4, lanes S and V), although the intensity of this signal was about 1% of that in flowers. The plant tissue used for these RNA isolations did contain a small number of early developing flowers[20], and it is possible that this low level of signal is the result of RNA in these developing flowers. We estimate the level of *AG* RNA to be ~10^{-4} in poly(A)$^+$ RNA of flowering apices, on the basis of both reconstruction experiments and the frequency of cDNA isolation.

To determine if *AG* expression is organ-specific, *in situ* hybridizations were performed. Sections of wild-type *Arabidopsis* flowers were prepared and probed with ^{35}S-labelled RNA synthesized from the *AG* cDNA template (Fig. 5). These results indicate that *AG* is expressed in stamens and carpels and not (or at much lower levels) in sepals or petals.

Discussion

We have isolated and characterized a flower-specific *Arabidopsis* homeotic gene, *AG*. The insertion mutant allele, *ag-2*, and the EMS-induced mutant allele, *ag-1*, both display alterations in the genomic region from which the presumed *AG* RNA is transcribed. The changes in the mutant alleles seem to interfere with normal transcription or splicing.

The deduced sequence of the AG protein shows striking similarity to the sequences of transcription factors from both humans (SRF, ref. 16) and yeast (encoded by *MCM1*, ref. 17, and *ARG80*, ref. 18), and to the deduced amino-acid sequence of a recently isolated homeotic gene (*def A*) from the flowering plant *Antirrhinum majus*[19]. In humans, the serum response factor (SRF) is thought to be required for the serum-inducible transcriptional activation of genes such as c-*fos*, a nuclear proto-oncogene of mammals[16]. In yeast, the product of the *MCM1* gene (GRM/PRTF) is a transcriptional regulator of mating-type-specific genes[21]. The region shared by these proteins spans ~56 amino-acid residues and, allowing for conservative substitutions, *AG* is ~80% similar in this region to all of these proteins (Fig. 3). This same region of SRF encodes the DNA-binding and dimerization domains[16,22]. On the basis of this sequence similarity and the *ag* phenotype we propose that the *AG* protein is a transcription factor involved in regulating genes that determine stamen and carpel development in wild-type flowers.

Like those of *Arabidopsis*, flowers of *Antirrhinum* consist of four concentric whorls of organs (sepals, petals, stamens and carpels). Mutations in the *Antirrhinum def A* homeotic gene cause the conversion of petals into sepals and stamens into carpels. This phenotype does not resemble that of *ag*, but closely resembles the phenotype of the *ap3* and *pi* mutants of *Arabidopsis*[3], suggesting that the *Antirrhinum def A* gene could be the homologue of either the *AP3* or *PI* gene of *Arabidopsis*. The *AG* gene product displays a high degree of similarity to DEF A in the DNA-binding region (Fig. 3), but not elsewhere. As the phenotypes of *Arabidopsis ag* and *Antirrhinum def A* mutants are very different, it seems likely that these genes function in completely different regulatory steps in flower development. The AG and DEF A proteins are more similar to each other than either is to the SRF, MCM1 and ARG80 proteins (Fig. 3).

We have recently isolated a family of other genes from *Arabidopsis*, some of which are preferentially expressed in flowers, that all share extensive sequence similarity to the DNA-binding regions of the SRF-MCM1 class of transcription factors (H.M., M.F.Y. and E.M.M., unpublished results). This indicates that there is a family of related genes that may act to control many of the steps in organ development in *Arabidopsis* flowers. This situation might be analogous to animal development where specific DNA-binding motifs have been adopted for different regulatory circuits. For example, *Drosophila* homeotic selector genes share the homeobox motif, the *Drosophila* gap genes all encode zinc-finger-type proteins, and genes involved in neurogenesis share the helix-loop-helix motif[23,24]. The MCM1[21] and SRF[25] gene products interact with other factors in regulating gene expression, and it is therefore possible that both AG and DEF A also interact with other factors to exert their regulatory control; some of these factors may be encoded by other homeotic genes known to exist in *Arabidopsis* and *Antirrhinum*.

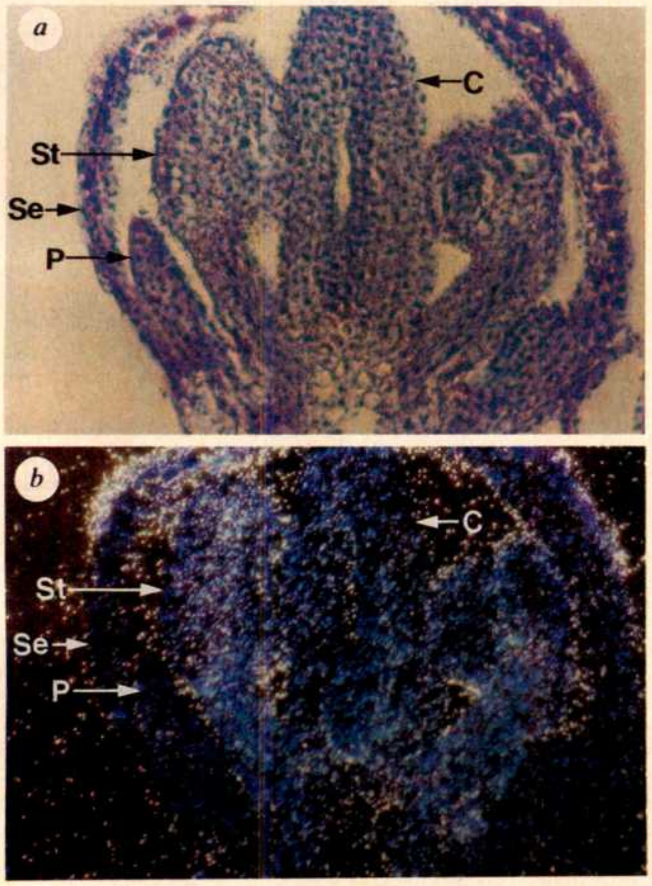

FIG. 5 *In situ* hybridization of *AG*. To determine the pattern of *AG* expression, ^{35}S-labelled antisense RNA was synthesized from an *AG* cDNA template (pCIT565), and hybridized to 8 μm longitudinal sections of stage-9 *Arabidopsis* flowers[3,20] as previously described[31]. *a*, Bright field and *b*, dark field. Arrows indicate the position of floral organs including Se (sepals), P (petals), St (stamens) and C (carpels). Hybridization of antisense RNA to the *AG* mRNA is localized to the positions of stamens and carpels and not to sepals and petals, indicating that it is in these organs that the *AG* gene is preferentially expressed. The signal over the upper portion of the sepals is observed both with sense and antisense RNA probes and therefore does not represent specific hybridization.

In addition, we used RNA blot analysis to identify the 1.1-kb *AG* mRNA and found it to be preferentially expressed in flowers. *In situ* hybridization experiments show that this RNA is localized to the stamens and carpels of wild-type flowers, the same organs that are lacking in *ag* mutants. It will be interesting to determine if the pattern of *AG* gene expression is altered in any of the other *Arabidopsis* homeotic flower mutants. ☐

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LETTERS TO NATURE

Detectability of γ-rays from clumps of dark matter

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If the dark matter in our Galaxy is made up of weakly interacting massive particles (WIMPs) with masses of the order of several GeV (for example, photinos or Higgsinos), γ-rays produced by their annihilation would in principle be observable[1,2]. But the expected flux[3] from a smoothly distributed dark matter halo[4,5] is much smaller than the observed diffuse background[6], and although narrow lines might be produced, their intensity would be much too low to see with the Gamma Ray Observatory (GRO)[3,7]. A complementary approach is to consider unique spatial signatures. Numerical simulations of galaxy formation[8] show that even in the central bulge of the Galaxy, the mean density of the dark matter could be equal to that of the stars. If this were so, GRO could see the Galactic Centre as a source of annihilating dark matter[1,3]. Other lumps formed as part of the hierarchical formation of the Galaxy could also produce sources that would be recognized by the shape of their continuum spectrum[2,3] and a line feature in sufficiently bright sources[3,7]. Even Geminga[9,10], the second strongest source of γ-rays at energies greater than 50 MeV, could be annihilating dark matter.

Lumps clearly formed during the hierarchical formation of the Galaxy. Clusters of galaxies are large-scale versions of this process, several per cent of their mass is contained in galaxian lumps with masses of $\sim 10^{-4} M_{\rm cluster}$ and density contrasts of $\sim 10^2$. As shown below the density contrast needs to be higher for the lumps to survive in the Galaxy, so I assume that a lower percentage of the mass is in such lumps. These lumps must either be the remnant cores of larger structures or have formed from non-gaussian initial conditions[11,12].

The dark matter in the halo has a local density[4,5] $\rho_{\rm h,0} \approx 10^{-2} M_\odot\,{\rm pc}^{-3}$. The distance to the nearest lump is $D_{\rm near} \approx (M_{\rm cl}/\eta\rho_{\rm h,0})^{1/3}$, where η is the fraction of the dark matter in lumps with cluster masses, $M_{\rm cl}$, of $M_6 \times 10^6\,M_\odot$. The flux of γ-rays with energies >50 MeV observed from this lump is

$$F = 5.8\times10^{-11}\,{\rm cm}^{-2}\,{\rm s}^{-1}(\sigma V)_{26}\left(\frac{X}{7}\right) m_{\lambda,5}^{-2} M_6^{-2/3} \times\left(\frac{\eta\rho_{\rm h,0}}{10^{-2}\,M_\odot\,{\rm pc}^{-3}}\right)^{2/3}\int_0^\infty \rho_{\rm cl}^2 r^2\,{\rm d}r \qquad (1)$$

where $(\sigma V)_{26}$ is the annihilation cross-section in units of $10^{-26}\,{\rm cm}^3\,{\rm s}^{-1}$, $m_{\lambda,5}$ is the WIMP mass in units of 5 GeV and the integral over the density in the lump, $\rho_{\rm cl}$ has units $M_\odot^2\,{\rm pc}^{-3}$. X, the average number of γ-rays with energies >50 MeV per annihilation, is fixed by e^+–e^- collider data[13] and is $7.1\,m_{\lambda,5}^{1/2}$. For the particular case of photinos[7] with masses >5 GeV

$$(\sigma V)_{26} \approx 0.1(\Omega_\lambda h_{100}^2)^{-1} m_{\lambda,5}^{-2} \qquad (2)$$

where h_{100} is the Hubble constant, H, in units of $100\,{\rm km\,s^{-1}\,Mpc^{-1}}$, and Ω_λ is the fraction of the closure density in WIMPs.

I adopt a density profile appropriate for lumps that have undergone mutual collisions and tidal shocking by the disk of the Galaxy[14]

$$\rho_{\rm cl} = \frac{\rho_0}{(1+r^2/r_c^2)^2}; \qquad \int_0^\infty \rho_{\rm cl}^2 r^2\,{\rm d}r = 2.3\overline{\rho_{1/2}}M_{\rm tot} \qquad (3)$$

where $M_{\rm tot}$ is the total mass, $\overline{\rho_{1/2}}$ is the mean density inside the half-mass radius, and r_c is a core radius.

The density $\overline{\rho_{1/2}}$ constrains the redshift of dissipationless formation for the lumps[15] and determines their ability to survive in the Galaxy. When galaxies form without dissipation, they reach maximum expansion and turn around when their density is a factor of 5.5 greater than the critical density $\rho_{\rm crit} = 3H^2/8\pi G$, where G is the gravitational constant. If pressure is unimportant, the protogalaxy collapses by a factor of two to become bound and virialized. Hence, the relationship between the redshift of turn-around, $z_{\rm turn}$ and $\overline{\rho_{1/2}}$ is

$$(1+z_{\rm turn}) = 43 h_{100}^{-2/3}\left(\frac{\overline{\rho_{1/2}}}{1\,M_\odot\,{\rm pc}^{-3}}\right)^{1/3}$$

The density $\overline{\rho_{1/2}}$ also determines whether the lumps survive mutual collisions and tidal shocking by the galactic disk. Using the impulse approximation, one can calculate the timescale for disruption owing to mutual collisions of uniform-density spheres of mass M_l, radius r and density ρ_l (see Appendix B of ref. 16). Equating M_l to M_{cl} and ρ_l to $\overline{\rho_{1/2}}$ leads to an underestimate of the disruption timescale for the more tightly bound lumps with density profiles given by equation (3). The fractional energy change during a collision with impact parameter $d \gg r$ and relative velocity v is

$$\Delta E/E = \frac{2GM_l^2}{3\pi d^4 v^2 \rho_l} \qquad (4)$$

During the age of the Universe t_U the closest encounter will have a typical impact parameter given by $d_{close}^2 = M_l/\pi v t_U \eta \rho_h$, where $\eta\rho_h$ is the density of the colliding objects. Because the energy change is dominated by the closest encounter

$$\Delta E/E = \frac{2\pi G t_U^2 \eta^2 \rho_h^2}{3\rho_l} = \frac{\eta^2 \rho_h^2}{27\rho_{crit}\rho_l} \qquad (5)$$

where the Freidmann equations with a cosmological density parameter of unity were used to replace t_U^2 with the critical density of the Universe ρ_{crit}. Adopting $\Delta E/E = 1$ as the critical value for survival versus destruction, the survivors must have densities

$$\rho_l > \frac{\eta^2 \rho_h^2}{27\rho_{crit}} = 13\eta^2\ M_\odot\ \text{pc}^{-3} \qquad (6)$$

When the density in the lumps satisfies this inequality, the dominant disruption mechanism becomes tidal shocking as they pass through the disk[17]. This has a timescale, $t_{shd} = 10^{10}$ $(\overline{\rho_{1/2}}/10 M_\odot\ \text{pc}^{-3})$ yr. With $\eta_{-2} = (\eta/10^{-2}) = 1$ and $\rho_{10} = (\overline{\rho_{1/2}}/10 M_\odot\ \text{pc}^{-3}) > 1$, the lumps survive collisions with one another and the disk. The nearest lump is at a distance $D_{near} \approx 10\ \text{kpc}(M_6/\eta_{-2})/^{1/3}$. In the general direction of the Galactic Centre, the flux is that expected from a smooth model of the halo.

The expected flux from the nearest lump is then

$$F = 6.8 \times 10^{-6}\ \text{cm}^{-2}\ \text{s}^{-1}\ m_{\lambda,5}^{-7/2} \eta_{-2}^{2/3} M_6^{1/3}$$
$$\times (\Omega_\lambda h_{100}^2)^{-1} \rho_{10} \left(\frac{\rho_{h,0}}{10^{-2}\ M_\odot\ \text{pc}^{-3}} \right)^{2/3} \qquad (7)$$

If z_{turn} were fixed rather than $\overline{\rho_{1/2}}$, there would be no dependence on the Hubble constant. The source brightness falls by a factor of two at a distance $R = 10\ \text{pc}(M_6/\rho_{10})^{1/3}$ from its centre, yielding an angular extent of ~3 arcmin. The freedom in choosing the factors in the product $m_{\lambda,5}^{-7/2} \eta_{-2}^{2/3} M_6^{1/3} \rho_{10}$ makes it impossible to predict source brightnesses. I have chosen values to yield a model where the brightest sources would appear in the Cos-B catalogue[18].

There are two immediate questions. Are there known lumps of dark matter that are potential sources? And are there any known γ-ray sources that might be identified with such lumps?

The extreme dwarf spheroidal satellites of the Milky Way, Draco and Ursa Minor, are good examples of dark lumps[19]. They are too distant to be detected, but their existence is difficult to reconcile with gaussian initial conditions[11]. Peebles[12] found that the Corona Cloud of galaxies is inconsistent with gaussian initial conditions. There are also two candidate 'dark clusters' with properties similar to those that I have assumed, one[20] with a distance of ~3.6 kpc, $M_6 = 0.5$ and $\overline{\rho_{1/2}} \approx 5 M_\odot\ \text{pc}^{-3}$ and another[21] with a distance of 5-8 kpc, $M_6 = 0.2$ and $\overline{\rho_{1/2}} \approx 3 M_\odot\ \text{pc}^{-3}$. Both of these clusters could be detected with GRO. Peebles[22] suggested that all globular clusters reside in dissipationless haloes with $M_6 \approx 100$ and $\rho_{10} \lesssim 1$, making them potential sources for GRO. Finally, I[23] invoked similar lumps so that the disk dark matter[24] could be the same as the halo dark matter.

As previously stated, several parameters have been chosen to yield fluxes comparable to the bright sources in the Cos-B catalogue. Among these, Geminga is an excellent candidate. It has an observed flux[10] of $4.8 \times 10^{-6}\ \text{cm}^{-2}\ \text{s}^{-1}$, making it the second strongest source of γ-rays with energies >50 MeV. Its angular size is <0.4°, the angular resolution of Cos-B. X-ray[25] and optical counterparts[26] have been tentatively identified with luminosity ratios $L_\gamma/L_X \approx 1{,}000$ and $L_X/L_{opt} \approx 1{,}800$. The current models are a binary pair of neutron stars[27] at a distance of <11 pc or a young pulsar at a distance of 1 kpc whose radio emission is beamed away and whose synchrotron nebulae is mysteriously absent[26]. The precise nature of this unusual source and its identification with the optical and X-ray counterparts remain uncertain. Geminga has a galactic latitude of only 4° which might make its association with the halo rather than the disk seem odd. But 9% of the total solid angle is within 5° of the disk, the surveys have concentrated on this region and the action of dynamical friction on lumps draws them toward the plane of the disk[28]. Nonetheless, some sources should be detected at high galactic latitude if this model is valid. The initial GRO survey with EGRET should accumulate 10^4 counts and determine Geminga's spectrum and spatial extent. The continuum spectrum is uniquely determined by the mass of the particle[1-3]. It will be possible to observe the narrow lines[3,7] with greater integration time or stacking of similar sources[29]. □

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Free electron density in H I regions as an indicator of ionizing dark matter

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It has recently been proposed[1] that the free electrons giving rise to the observed dispersion of pulsar signals result mainly from photoionization induced by the decay of dark matter particles in the Galaxy. For reasons related to conservation of energy and momentum the decay photons would have a unique energy in the rest frame of the decaying particles, and in the proposed theory

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this energy is close to 14 eV. A crucial prediction of the theory is that in regions where the dark matter density is approximately constant, the free electron density n_e in opaque H I regions should also be approximately constant, when averaged over several mean free paths for the ionizing photons. The determination of an accurate distance for the pulsar PSR1451-68 (ref. 2), in combination with its known dispersion measure and with previous observations of two other pulsars with accurate distances, leads to a successful test of this prediction.

The dark matter particles concerned are likely to be neutrinos of non-zero rest mass. It is well known that neutrinos of mass ~30 eV created by pair production in the Big Bang would possess the critical cosmological density if the Hubble constant is ~50 km s^{-1} Mpc^{-1}. Such a mass is now ruled out for electron-type neutrinos but not for μ- and τ-types. In my theory[1] the decaying neutrinos turn out to have a well defined mass m_1 of this order, 27.7 ± 0.5 eV. Such a neutrino would be expected[3] to decay into a photon and a neutrino of another type and lower rest mass m_2. If $m_2 \ll m_1$ the energy of the decay photon would be $\sim\frac{1}{2}m_1$, here ~13.85 ± 0.25 eV. Such a photon would just be able to ionize hydrogen.

The flux of these decay photons depends on the concentration n_ν of neutrinos and on the lifetime τ for their decay. The concentration can be deduced from the observed rotation velocity of the Galaxy, and is $n_\nu \approx 5\times10^7$ cm^{-3} (ref. 1). For a decay photon energy of ~14 eV the interstellar medium would be highly opaque (for a neutral hydrogen density of ~0.1 cm^{-3} the mean free path is ~1 parsec), and the photoionization equilibrium is then simply

$$\frac{n_\nu}{\tau} = \alpha n_e^2 \qquad (1)$$

where α is the appropriate recombination coefficient. If this mechanism is to account for the value of n_e implied by observed pulsar dispersion measures, ~0.05 cm^{-3} in H I regions near the Sun[4], τ must be ~10^{23} s (ref. 1). The standard electroweak model gives[3] a much larger value of τ, but various non-standard models are under investigation[5,6] which could give the required value.

Here I am primarily concerned with the value of n_e within a few hundred parsecs of the Sun, but will first mention the implications of equation (1) for more distant regions of the Galaxy. The recombination coefficient is expected to be fairly constant; it depends on the kinetic temperature T of the electrons, approximately as $T^{-1/2}$. Therefore, $n_e \approx T^{1/4}$, a weak dependence. Moreover, in the heating regime of concern, T would not be expected to deviate from 10^4 K by more than a factor of about two in the intercloud medium over large regions of the Galaxy[7]. I can therefore assume that α is constant.

I now consider the variation of n_e with galactocentric radius r and height z above the galactic plane. Following the analysis of the pulsar data by Lyne, Manchester and Taylor[8], $n_e \approx 1/(1+r/a)$, where the core radius a is $\sim r_\odot$. Equation (1) then implies that $n_\nu \approx 1/(1+r/a)^2$. This leads to a test of the theory, because the r-dependence of n_ν influences the rotation curve of the Galaxy. In fact, there is good agreement with the observed rotation curve[9]. As regards the dependence of n_e on z, one would expect to find an essentially constant n_e at heights less than the scale height of the neutrino distribution. But this would clearly cease to be true at the height where the total gas density becomes less than the constant value of n_e implied by equation (1). Above this height the gas must cease to be opaque, and the assumption of local photoionization equilibrium would break down. Above this point n_e would be essentially equal to the total gas density. Recent observations of the dispersion measure of pulsars in globular clusters up to 20 kpc above the plane indicate that this transition layer occurs at $z \approx 600$ pc (A. G. Lyne, P. Salucci and D.W.S., manuscript in preparation).

In the region a few hundred parsecs from the Sun, one would expect n_ν to be approximately constant and τ to be exactly constant, being determined by considerations of elementary-particle physics. Thus n_e should be independent of position in opaque regions near the Sun, when averaged over several mean free paths, that is, over several parsecs. By contrast, if the ionizing photons were produced by the relatively widely separated O or B stars, and were subject to complex and position-dependent absorption effects, one would expect to find that the n_e in H I regions derived from the pulsar dispersion-measure data differ for the line segments to different pulsars.

Two of the pulsars for which the free electron densities are known and for which accurate distances have been determined from parallax measurements, PSR0950+08 and PSR0823+26, were recently discussed by Reynolds[4]. The line segments involved are separated by ~28°, and do not intersect any known H II regions. The inferred mean values of n_e were 0.023 cm^{-3} and 0.054 cm^{-3}, respectively. Reynolds[4] pointed out that a correction is necessary for the part of the line of sight that passes through the low-density bubble[10] immediately surrounding the Sun. This bubble contains X-ray emitting gas at a temperature of ~10^6 K and at a correspondingly low density. The correction is important for the first pulsar and Reynolds[4] concluded that the gas responsible for its dispersion measure must be confined primarily to the outer 47 pc of the line segment, leading to an electron density in that region of ~0.056 cm^{-3}. By contrast, the correction for the second pulsar is small. Thus essentially the same value of n_e in normal H I regions was derived for both line segments, ~0.05 cm^{-3}. Reynolds also concluded, from a detailed study of the O and B stars near these line segments, that these stars were probably not mainly responsible for the ionization along the segments, but could have contributed ~10–20% of the observed ionization.

Using the newly determined[2] distance for PSR1451−68 (450 ± 60 pc) and the dispersion measure, the electron density is 0.019 ± 0.003 cm^{-3}. This value is much smaller than 0.05 cm^{-3}, and correction for the local bubble (the radius of which is <50 pc in the direction of this pulsar)[10] would not make much of a difference. My hypothesis thus requires another suitably large bubble along the line of sight to this pulsar. The galactic coordinates of PSR1451−68 are $l \approx 315°$, $b \approx -8.5°$. This line of sight passes right through loop I, a well-known nearby supernova remnant. The position and geometry of this loop are clearly shown in Fig. 2 of ref. 10. To correct for the presence of this loop, I assume a path length of 300 pc, and adopt $n_e \approx 10^{-2}$ cm^{-3} (refs 11, 12) along this segment. This low density is associated with the high temperature of this X-ray emitting region. The contribution to the dispersion measure is therefore ~3 cm^{-3} pc. As the observed dispersion measure is 8.6 cm^{-3} pc, I conclude that the final ~100 pc of path length through a normal H I region[13] to the pulsar contributes ~5.6 cm^{-3} pc. Thus n_e in the section of the path through the normal H I region ~0.056 cm^{-3}, in good agreement with the corresponding quantity for the first two pulsars with well determined distances, and with the prediction of my theory.

The most direct observational test of the hypothesis would be to search for an unidentified line in the background at an energy of ~14 eV. This line would be produced by decaying particles in the immediate vicinity of the Sun, so that its strength F can be predicted only in relation to an understanding of the local interstellar medium. This problem is discussed elsewhere[14], where I obtained the estimate $F \approx 2\times10^3$ cm^{-2} s^{-1}.

The necessary narrow-band observations in the far ultraviolet have not so far been carried out. The upper limit for the flux of a line at ~14 eV derived from the broad-band observations of Holberg[15] is ~3×10^4 cm^{-2} s^{-1}, which is compatible with my estimate.

An attempt to search for a line in the background in the vicinity of 14 eV is being planned in collaboration with S. Bowyer and R. Stalio. If it is discovered at the flux level indicated here, it would place the hypothesis of decaying dark matter on a firm footing. □

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Discovery of two radio pulsars in the globular cluster M15

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WE report the discovery of two radio pulsars, 2127+11B and 2127+11C, in the globular cluster M15 (NGC7078), which also contains the 110-ms pulsar 2127+11A (ref. 1). Although only twenty globular cluster pulsars are known at present, the detection of three pulsars in a single cluster suggests that there might be a large total population of these objects, which would make them powerful probes of the dynamics and evolution of globular clusters. One of the new pulsars, 2127+11C, is in a highly eccentric binary system with an 8-hour period. It is thus similar to the famous PSR1913+16 system[2], and study of the pulse arrival times can be expected to provide tests of general relativity, including gravitational wave emission. The companion of PSR2127+11C is probably either another neutron star or a massive white dwarf, suggesting that the core of M15 contains a high density of massive stellar remnants.

The initial observations were made using the 305-m Arecibo radio telescope at a central frequency of 430 MHz and a 10-MHz receiver bandwidth. The data were sampled at an effective rate of 1.974 kHz using the Arecibo 40-MHz three-level correlation spectrometer in a manner identical to that used in the discovery of PSR2127+11A (ref. 1). The discovery observations were made on 26 December 1988 starting at 18:51 UT. The pulse search was carried out on the NCUBE/10 (California Institute of Technology), a concurrent computer having 512 central processing units interconnected with hypercube topology. Assuming a dispersion measure of 67.25 pc cm^{-3}, the measured value[1] of PSR2127+11A, a de-dispersed time series of 2^{24} points was formed by supplementing the observed 10.9 million samples with the mean value. The power spectrum was calculated using an implementation of the fast Fourier transform (FFT) algorithm for concurrent computers[2] and was searched for families of significant harmonic peaks.

Table 1 and Fig. 1 give the parameters and pulse profiles of the three pulsars detected in the 430-MHz observations. PSR2127+11A was clearly detected in our first analysis of the December 1988 data. Further inspection revealed PSR2127+11B with a period of 56 ms, about twice as faint as PSR2127+11A. Subsequent timing measurements spanning 230 days indicate that PSR2127+11B is an isolated pulsar with a period derivative $\dot{P}$ of 9×10^{-18} s s^{-1}, implying a characteristic age of 1×10^{8} yr, if the observed $\dot{P}$ is due solely to magnetic braking. If, on the other hand, one assumes that the dominant effect is acceleration due to the local gravitational field, as in the case of PSR2127+11A, then the magnitude of the acceleration is 5×10^{-8} m s^{-2}. The errors quoted for PSR2127+11B in Table 1 are three times the formal errors given by the temporal fitting program used in the analysis (TEMPO). The accuracy of these measurements are limited by the coupling of the timing residuals owing to a position error (a sine wave of period one year) and a $\dot{P}$ error (quadratic drift) over the limited observation period of 230 days.

PSR2127+11C was discovered using an algorithm specifically developed for detection of pulsars in binary orbits. The variable Doppler shift due to the orbital acceleration can spread the signal power over multiple frequency bins, resulting in a loss in sensitivity, particularly for fundamentals or harmonics with millisecond periods. In our algorithm, samples were inserted or deleted in the time series to compensate for a presumed acceleration. To reduce the computational demands of the search, we assumed a constant acceleration. A range of accelerations from -19.2 m s^{-2} to $+19.2$ m s^{-2} was explored using 2^{24}-point transforms, corresponding to the acceleration experienced by a pulsar in a circular orbit with 1-d period and a 1-$M_\odot$ companion. A series of 2^{22}-point transforms was also calculated for accelerations up to 203 m s^{-2}, corresponding to a 1-$M_\odot$ companion and a 4-h circular orbit. PSR2127+11C, with a period of 30.5 ms, was initially found in a 2^{24}-point transform at an acceleration of -9.5 m s^{-2}.

Timing measurements were begun to determine the keplerian parameters of the PSR2127+11C orbit. Recording of the raw data stream on magnetic tape allowed us to extract timing information for all three pulsars from the same data. Because of the faintness of PSR2127+11C, an integration time of $\geqslant10$ minutes was required for detection. This necessitated an acceleration search for each observation to compensate for orbital motion and to obtain the correct apparent pulsar period. The determination of the orbital period was also complicated by the limited duration (3 h) over which the cluster can be observed by the Arecibo telescope. The orbital period was found to be 8.05 h, indicating that it would take a minimum of 6 weeks for a complete cycle of observable orbital phase. In Fig. 2, we display the apparent pulsar period at the midpoint of each observation as a function of the orbital phase for observations taken primarily over an 8-week period. The eccentricity of the orbit is immediately apparent.

Using the velocity curve in Fig. 2 for an initial estimate of the orbit, we analysed the pulse arrival time on 17 observations taken during May 1988. The resulting keplerian parameters, surprisingly similar to those of PSR1913+16 (ref. 2), are given

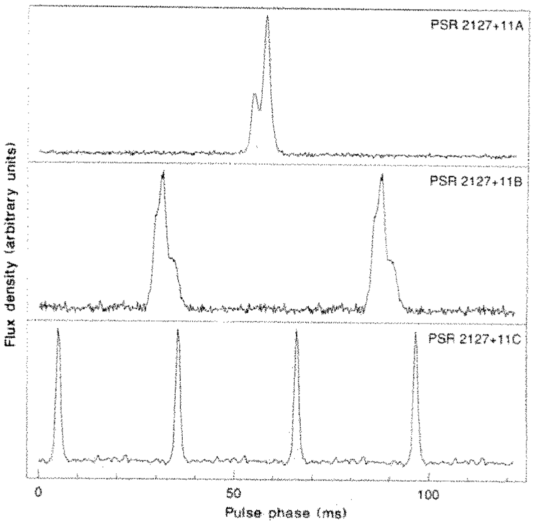

FIG. 1 Average pulse profiles for the three known radio pulsars in globular cluster M15. The time resolution is 500 μs.

TABLE 1 Pulsar parameters

Timing parameters		2127+11A	2127+11B	2127+11C
Epoch (JD)	T_0	2,447,213.15	2,447,632.9906	2,447,660.9089
Pulse period (ms)	P_0	110.66470954 (1)	56.13303358 (1)	30.529 (1)
Period derivative (10^{-18} s s^{-1})	$\dot{P}$	−20 ± 1	8.8 ± 1.1	—
Right ascension (1950.0)*	α_{m15}	−1.95 ± 0.15″	+3.86 ± 0.15″	—
Declination (1950.0)*	δ_{m15}	+0.6 ± 0.3″	−0.8 ± 0.6″	—
Dispersion measure (pc cm^{-3})	DM	67.25 ± 0.05	67.25 ± 1.0	67.25 ± 1.0
Flux density (mJy)	S_{430}	1.7 ± 0.4	1.1 ± 0.4	0.6 ± 0.2
Orbital period (s)	P_b	—	—	28,969 ± 1
Projected semi-major axis (light s)	$a_1 \sin i$	—	—	2.52 ± 0.01
Eccentricity	e	—	—	0.680 ± 0.003
Longitude of periastron (deg)	ω_0	—	—	316.4 ± 1.0
Epoch of periastron (JD)	T_0	—	—	2447632.46720 (5)
Mass function ($M_\odot$)	f	—	—	0.15

Timing parameters for PSR2127+11A, B and C. The values given for PSR2127+11A are those reported in Wolszczan *et al.*[1] and are consistent with the results of our 430-MHz observations. The values for PSR2127+11B are based on 430-MHz observations spanning 230 days. The keplerian orbital parameters for PSR2127+11C are based on 17 days of pulse arrival timing. Numbers in parentheses indicate the uncertainty in the last significant figure.

* Positions relative to the centre of globular cluster M15 ($\alpha = 21^h\,27^m\,33.35^s$, $\delta = 11°\,56'\,48.8''$)[16].

in Table 1. If the secondary is another neutron star, the estimated orbital decay time and the advance of periastron will be 2×10^8 yr and 4° yr^{-1}, similar to those of PSR1913+16. The PSR2127+11C system should thus provide tests of general relativity in the strong-field limit, including gravitational wave emission, as does PSR1913+16. The errors quoted in Table 1 are our best estimates of the uncertainty arising from the limited time span of observations. A significantly more accurate measurement of the pulsar parameters, including a precise determination of the advance of periastron, is in progress using approximately nine months of timing observations.

Constraints can be placed on the pulsar and companion masses (M_p and M_c) by determining the mass function f, which depends on the apparent size of the pulsar's orbit and the orbital period. The value of the mass function for the 2127+11C system is $f(M_p, M_c) = (M_c \sin i)^3/(M_p + M_c)^2 = 0.15 M_\odot$. Assuming $M_p = 1.4 M_\odot$ for PSR2127+11C, the minimum mass of the companion is 0.94 $M_\odot$. For $M_p = M_c = 1.4 M_\odot$, the inclination angle i of the orbital plane to the line of sight would be 49°, close to the median inclination angle of 60°. Given the measured orbital parameters, the distance of closest approach is of the order of $R_\odot$ for inclination angles corresponding to a 1-$M_\odot$ companion. A main-sequence companion is therefore ruled out, and the companion is most likely a second neutron star or a massive white dwarf.

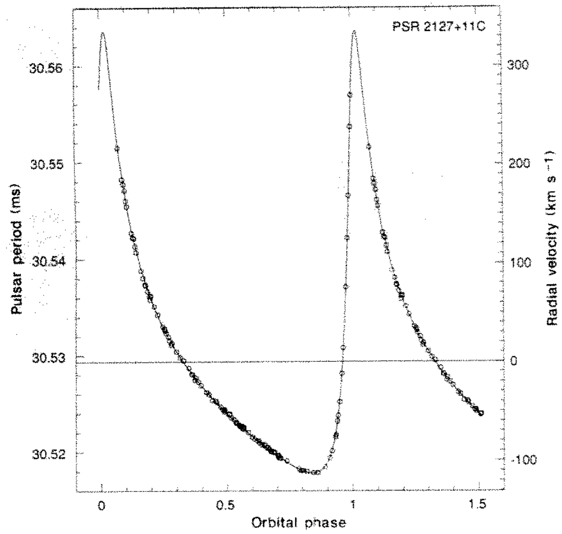

FIG. 2 Velocity profile and observed Doppler-shifted pulse periods as a function of orbital phase for PSR2127+11C. The solid line is the apparent pulse period derived from the five keplerian orbital parameters given in Table 1.

The discovery of three pulsars in a single cluster suggests that pulsars are abundant in globular clusters. Kulkarni *et al.*[4] have quantitatively analysed the results of the major surveys of cluster pulsars and conclude that a typical rich cluster contains about 100 active pulsars, most of which will be undetectable owing to the great distances to the clusters. Assuming a distance of 10 kpc to M15, the radio luminosities of PSR2127+11A, B and C are respectively 170 mJy kpc^2, 110 mJy kpc^2 and 60 mJy kpc^2. These luminosities are well above the minimum luminosity of 1 mJy kpc^2 for field pulsars. Hence with increased sensitivity, other pulsars ought to be detectable in M15. We are now analysing all our data (>50 h of observations) in an effort to locate fainter pulsars.

The discovery of the binary pulsar, 2127+11C strongly suggests an abundance of massive stellar remnants in the core of M15. According to the standard scenario, in some previous phase, PSR2127+11C must have been in a binary system with a non-degenerate companion. We rule out 2127+11C being a primordial binary because the formation of such systems is inevitably associated with a large systemic motion ($\gtrsim$200 km s^{-1})[5-7] which would have resulted in the expulsion from the cluster. Consequently, the companion is probably the result of a capture interaction. Alternatively, PSR2127+11C could have been a solitary spun-up pulsar (like PSR2127+11B) that experienced an exchange collision with a binary system. The large observed eccentricity indicates that an exchange may have taken place recently.

Various authors have argued[8-10] that accretion-induced collapse (AIC) of white dwarfs can produce pulsars. AIC scenarios invoke massive C+O white dwarfs or O-Ne-Mg white dwarfs and thus, even if AIC mechanism were to be operative (see Verbunt *et al.*[11] for a discussion of the considerable theoretical and observational difficulties with this mechanism), the discovery of PSR2127+11C implies an abundance of these white dwarfs. Such white dwarfs evolve from massive main-sequence stars ($M \leq 8 M_\odot$). Thus in the framework of either the standard or the AIC model we conclude that, in the past, M15 contained many massive stars. To achieve reasonable rates for exchange or capture interactions, the remnants of these massive stars must have aggregated in the core of M15 by mass segregation. Even taking this effect into account, very steep initial mass functions ($\propto M^{-\alpha}$, $\alpha \geq 3.5$) are ruled out for M15 because they predict insufficient numbers of massive white dwarfs and neutron stars. A flatter initial mass function also explains the large abundance of pulsars inferred from other observations[4]. We note, however, that some recent theoretical studies[12] suggest steep initial mass functions for most clusters that have survived to the present.

Most tidal-capture binaries (those captured by transfer of kinetic energy to stellar deformation) are expected to be formed with an orbital radius of $\sim R_\odot$, and thus an orbital period of a fraction of day. A small fraction (10–30%) are expected to be wide binaries ($P_{\text{orb}} \approx 100$ d) formed by capture of giants. The formation of single pulsars like 2127+11A and B is easily understood as resulting from the breakup of a wide binary by passing stars[13,14]. Rappaport *et al.*[15] have shown this to be a very effective mechanism for breaking up wide binaries in the especially high-density collapsed core of M15. The tidal interaction model predicts that there should be 3–10 times more compact binaries than isolated or wide-orbit binaries, whereas we detected only one compact binary and two isolated pulsars. Selection effects may have prevented detection of a larger number of short-orbital-period systems in our survey and a quantitative analysis of them will be presented elsewhere. ☐

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Coexistence of two liquid crystalline phases in poly(γ-benzyl-α, L-glutamate) solutions

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IN 1956, Flory[1] proposed a phase diagram for rod-like molecules of the form shown in Fig. 1. Since then, most of the major features of this diagram have been verified experimentally[2]. In particular, the two-phase 'chimney' is now well established. One feature that has not previously been established, however, is the slight maximum (the 'cap') in the phase boundary on the high-concentration side of the chimney. Flory[1] pointed out that this cap should manifest itself as two coexisting liquid-crystalline (anisotropic) phases. Here we provide evidence for bulk phase separation into two such phases in a macroscopic (~0.5 g) liquid-crystal sample held in a constant-temperature bath. A distinct boundary can be seen between the two phases, and they are distinguished further under an optical polarizing microscope.

Benzyl alcohol solutions of the synthetic polypeptide poly(γ-benzyl-α, L-glutamate), chosen as a model rod-like molecule, gel at ~60 °C. Details of sample preparation can be found elsewhere[3].

We have previously found[4] evidence for separation of the gel into two anisotropic phases at ~100 °C. In that experiment a small sample was heated in a hot stage so surface effects may have been significant and the phases may not have been in equilibrium. Here we immersed small-bore test tubes containing ~0.5 g of 15% gel (molecular weight 248,000) in a constant-temperature bath. Macroscopic phase separation (with an easily visible boundary) was achieved at temperatures ~80 °C after a day or so. A room-temperature photograph (Fig. 2, left) shows a tube with the two phases clearly visible. (Cooling rapidly to room temperature does not affect the appearance of the phase separation, but the quality of the photograph is better because it is not necessary to photograph through the hot oil.)

Examination of samples of the upper and lower phases in an optical polarizing microscope shows that both are anisotropic. The lower phase is strongly coloured and shows definite 'fingerprinting'[5] with a periodicity of ~15 μm (Fig. 2, lower right). The upper phase takes the form of a black and white speckle (Fig. 2, upper right). The two phases are clearly distinct; this is clear evidence for the equilibrium coexistence of two liquid-crystalline phases.

The phase separation takes about a day, that is, the boundary takes about a day to form. Given that the samples consist of 0.5 g of a highly viscous material, it is not surprising that a large-scale separation should take so long to occur. To find the lowest temperature at which the phase separation occurs, samples were heated at a rate of ~1 °C per day. The first separation was observed at 71 °C and examination of the lower phase showed that it was both coloured and characterized by 'fingerprints'. Previously we saw formation of the coloured phase at higher temperatures, but these results are not necessarily inconsistent because in the first experiment the heating rate was much faster, 2 °C min^{-1}, and the sample was much smaller. In the first experiment we observed the coloured phase immediately upon formation whereas in the experiments reported here it is not observed until bulk phase separation has occurred.

Russo and Miller[6] have seen evidence for the cap in the temperature dependence of fingerprint spacing at fixed concentration. As they reduced the temperature, the fingerprint spacing decreased and then at a certain temperature it began to increase again. From this they inferred that at this temperature the sample passed from the one-phase region to the two-phase cap. They did not actually see the second phase.

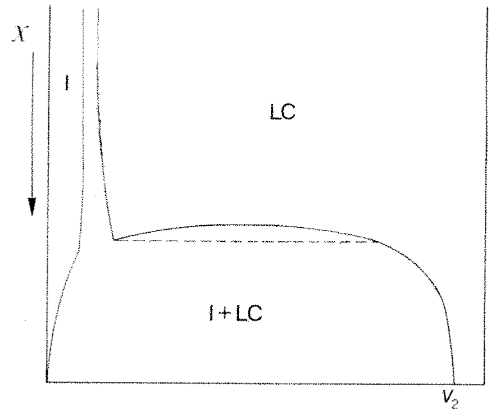

FIG. 1 The phase diagram of rod-like molecules as described by Flory[1]. The axes are quality of solvent, χ ($\approx cT^{-1}$, where T is temperature and c is a constant) and the volume fraction of polymer, v_2. The dotted line indicates the lower edge of the 'cap'. I, isotropic; LC, liquid crystalline.

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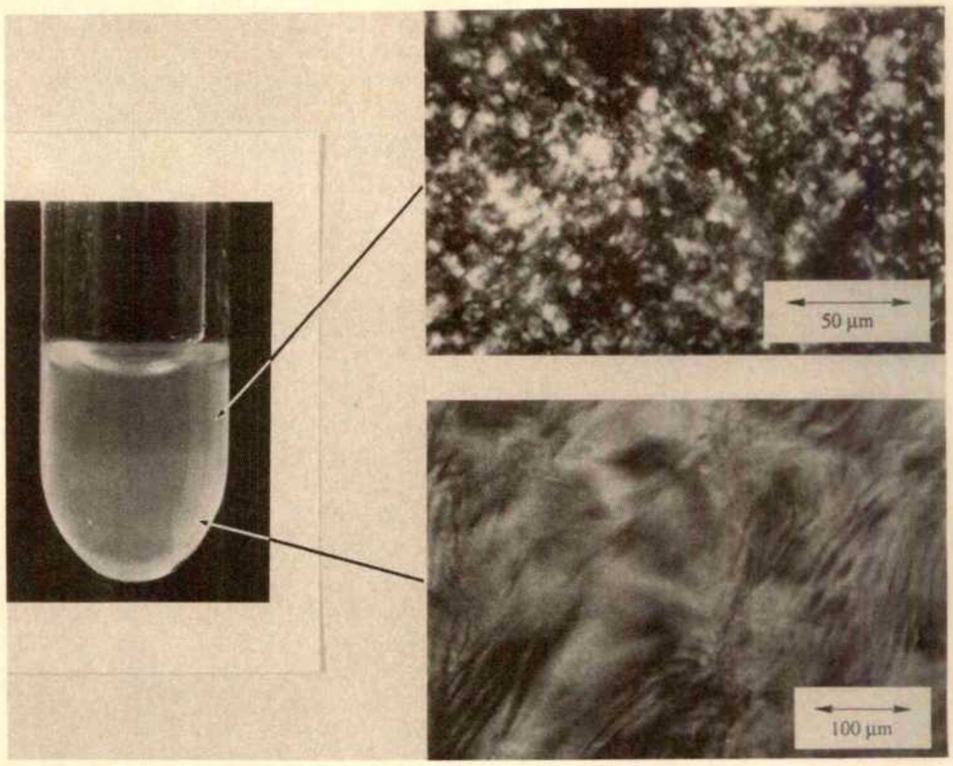

FIG. 2 Left, room-temperature photograph of the upper and lower phases, shown in the tube used for separating them. Right, the upper and lower phases seen between crossed polar filters.

The solvent used here was described by the supplier as 99% benzyl alcohol and was used as supplied. Gels were also made up with anhydrous benzyl alcohol (quoted as containing <0.005% water and supplied under nitrogen) and 99% benzyl alcohol that had been vacuum distilled in the laboratory and stored over a molecular sieve. No sample made with either of these latter solvents showed any sign of phase separation at any temperature. This can be attributed to the reduction of the solvent quality by water. Russo and Miller[7] have analysed the effect of a non-solvent on the Flory phase diagram and, for simplicity, displayed the results on a binary phase diagram rather than a ternary one. They found that the phase boundary is moved up for increasing amounts of non-solvent and also that the shape of the cap is altered slightly. The trace water in our system seems to lead to a greater range of temperatures between the gel's melting point (~60 °C, ref. 3) and the one-phase region, LC, and hence a larger two-phase region in which phase separation is possible once the crystals have melted. To verify this, we made up a sample of gel using anhydrous benzyl alcohol that had been deliberately contaminated with water (2% volume by volume). Again we observed phase separation, confirming that trace amounts of water shift the position of the phase diagram relative to the gel melting point and make the phase separation possible. Phase separation in the 'dry' system cannot be achieved because the melting point of the crystals (and hence the gel) is inside the single-phase region. Phase separations involving the isotropic phase (in different parts of the phase diagram) are now being investigated. □

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Depletion of H_2O_2 in a Greenland ice core: implications for oxidation of volcanic SO_2

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MAJOR volcanic eruptions inject large quantities of sulphur compounds into the atmosphere[1]. A principal component is sulphur dioxide; subsequent oxidation of SO_2 in the atmosphere to sulphuric acid aerosol may lead to climate perturbations[2]. Here we present evidence to suggest that SO_2 oxidation may occur at high latitudes by reaction with H_2O_2. Variations in sulphate and H_2O_2 concentrations in four sections of a Greenland ice core (corresponding to four volcanic events) show that high sulphate concentrations, from volcanic fallout, are accompanied by depletion of H_2O_2. This suggests that some of the volcanic sulphur was still in the form of SO_2 when it reached Greenland, and was then oxidized by H_2O_2 in Greenland precipitation. As SO_2 oxidation in the stratosphere is very slow, volcanic events that inject SO_2 into the stratosphere could produce large depletions of distant H_2O_2 reservoirs when the material is later reinjected into the troposphere.

During volcanic eruptions sulphur compounds, predominantly H_2S and SO_2, are released into both the troposphere and stratosphere. Once in the atmosphere, H_2S is very rapidly converted into SO_2 (ref. 3). Sulphur dioxide is then oxidized to sulphate and sulphuric acid particles by reactions occurring in the gas phase, in the aqueous phase, and/or on aerosol surfaces[4–6]. The sulphate aerosol plays a significant part in short-term climate change after volcanic eruptions, by scattering and absorbing solar radiation[7], which results in surface temperature cooling[8,9]. In fact, small but sulphur-rich eruptions may produce climate changes similar to or even larger than much more voluminous silicic, sulphur-poor eruptions[8,10,11]. As sulphur compounds are released in a reduced or incompletely oxidized form, an accurate knowledge of the production rate of sulphate aerosol from SO_2 is important for assessing the climate impact. In the aqueous phase, H_2O_2 is believed to be the most efficient oxidizing agent of SO_2 because, unlike most other species, oxidation kinetics of SO_2 by H_2O_2 are only slightly slowed down by a decreasing pH in an open system[12,13]. Here we present a detailed record of H_2O_2 and SO_4^{2-} concentrations in four volcanic acidity layers from an ice core drilled in 1985 at Site A, central Greenland (71° N, 36° W).

Solid electroconductivity measurements were performed to locate high-conductivity layers, which presumably represent high-H^+ layers associated with volcanic events[14]. For chemical analyses we sampled these high-conductivity layers, as well as adjacent parts of the core corresponding to a period of 3 to 4 years before and after each eruption, at intervals of 4-7 cm (~2 months) using ice-cleaning procedures similar to those described by Spencer *et al.*[15]. Anion (Cl^-, NO_3^-, SO_4^{2-}) and cation (Na^+, NH_4^+, K^+, Mg^{2+}, Ca^{2+}) concentrations were measured by ion chromatography. We determined the peroxide content of the samples using the fluorometric technique originally developed by Guilbault *et al.*[16] and later modified by Lazrus *et al.*[17]. For these samples, no distinction has been made between organic and inorganic peroxides so that all values for 'peroxide' in the samples refer to total inorganic and organic peroxides. The precision is of the order of 10% for ion chromatography measurements and 7% for peroxide measurements.

A preliminary depth-age relationship for the ice core was determined using density measurements and snow accumulation rates[18]. Comparisons with particle concentration measurements in a second core drilled at site A in 1985 (E. Mosley-Thompson, personal communication) confirmed that the high-acidity layers at 22.6, 36, 70.5 and 80.5 metres in the core correspond to the eruptions of Hekla (1947), Katmai (1912), Tambora (1815) and Laki (1973), as listed in Table 1. Variations in SO_4^{2-} and H_2O_2 as a function of depth for these four volcanic eruptions are shown in Fig. 1. Sulphate values have been corrected for the sea-salt contribution[19] and thus represent the non-sea-salt sulphate only.

Seasonal variations in the Cl:Na ratio in polar snow have been observed[19] and we used these to identify summer periods (Fig. 1). Both SO_4^{2-} and H_2O_2 exhibit seasonal variations in polar snow with peaks in the summer[19,20]. At depth, seasonal variations in the peroxide profiles are less clear (Fig. 1) because of the diffusion of the peroxide in the ice[21]. Significant enhancements in SO_4^{2-} concentrations in the Greenland ice are observed

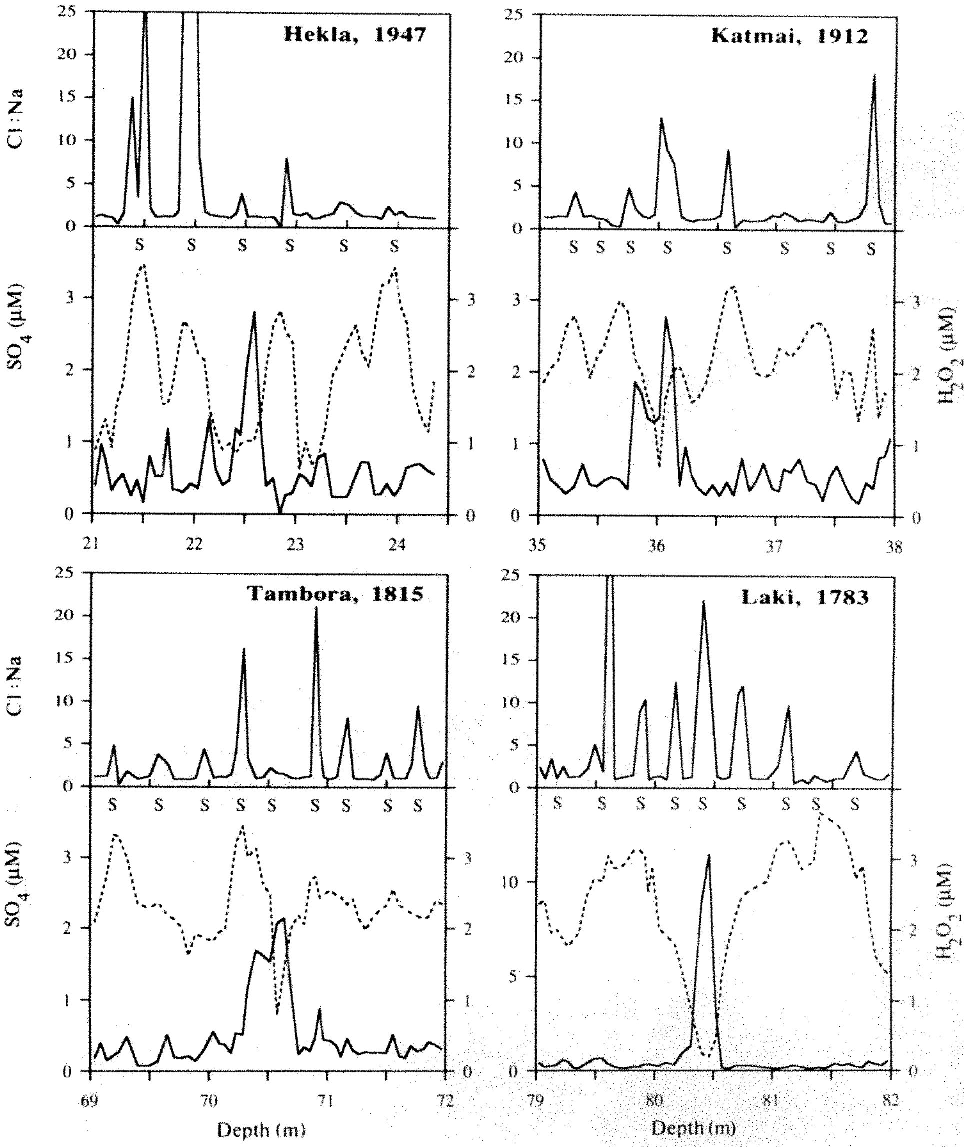

FIG. 1 Variation of non-sea-salt SO_4^{2-} concentration (—) and H_2O_2 concentration (----) as a function of depth for four sections of the Site A, Greenland ice core. Summer periods, based on variation of Cl:Na ratio are indicated by S.

TABLE 1 Sulphur conversion rates and chemical lifetimes

Volcanic events	SO_2 degassed* ($\times 10^{10}$ kg)	H_2O_2 loss (μmol l^{-1})	SO_4(volc) (μmol l^{-1})	%SO_2 (%)	Travel time (d)	Conversion rate ($\times 10^{-1}$ % d^{-1})	Lifetime (d)
Hekla 1947 Iceland	0.5	0.8	1.1	70	2-6†	0.7-2	5-15
Katmai 1912 Alaska	1-3	0.65	1.25	50	5-10‡	0.7-1.6	6-15
Tambora 1815 Indonesia	20-40	0.2	1.35	15	100-200§	0.05-0.1	100-200
Laki 1783 Iceland	15-20	2.1	6.0	35	2-6†	2-6	2-5

* From ref. 12.
† C. Weaver, personal communication.
‡ From ref. 27.
§ From ref. 26.

owing to the deposition of volcanic sulphuric acid, with an enrichment of up to twenty times the background for the 1783 Laki eruption. For all of the volcanic events, a distinct deviation from the expected seasonal peroxide profile is evident and very strongly correlated with increased excess SO_4^{2-} attributable to the volcanic events. This pattern is observed both for high-latitude (Laki, Hekla and Katmai) and low-latitude (Tambora) eruptions although these eruptions were chemically different[11,22] and took place at different times of the year. The depletion of H_2O_2 associated with increased SO_4^{2-} indicates that part of the volcanic sulphur was present as SO_2 over Greenland at the time the precipitation was forming, and that a reaction occurred between H_2O_2 and SO_2 in the supercooled water film at the surface of ice crystals according to the following mechanism[23]

$$SO_2 + H_2O \Leftrightarrow SO_2 \cdot H_2O \tag{1}$$

$$SO_2 \cdot H_2O \Leftrightarrow HSO_3^- + H^+ \tag{2}$$

$$HSO_3^- + H_2O_2 \Leftrightarrow HOOSO_2^- + H_2O \tag{3}$$

$$HOOSO_2^- + H^+ \rightarrow H^+ + HSO_4^- \tag{4}$$

$$HSO_4^- \Leftrightarrow SO_4^{2-} + H^+ \tag{5}$$

Although we cannot rule out the possibility of H_2O_2 destruction catalysed by volcanic dust particles, all these eruptions show the same pattern despite differences in the amount of dust that reached Greenland[24]. Furthermore, simulation of atmospheric HO_2 concentrations after the El Chichon eruption[7] indicate that HO_2 concentration changes in the volcanic plume are insufficient to account for the H_2O_2 depletion. Examination of the Cl:Na ratio establishes that the acid deposition took place for a period of one year or more following the Tambora and Katmai events. However, H_2O_2 decreases are only observed during summer. Thus, the SO_2-H_2O_2 reaction appears to take place only during the summer. In the Laki layer, the extensive SO_2 emission may have completely depleted peroxide in the precipitation.

To estimate conversion rates of SO_2, we have made the assumption that all of the peroxide depletion is the result of the reaction with SO_2. According to the reaction mechanism described above, a net loss of H_2O_2 increases SO_4^{2-} concentration by the same amount. By comparing the SO_4^{2-} produced from SO_2-H_2O_2 oxidation (H_2O_2 (loss)) and the total increase in SO_4^{2-} above background (SO_4^{2-} (volc)) we can estimate the proportion of sulphur still existing as SO_2 before the SO_2-H_2O_2 reaction took place in the precipitation as

$$\%SO_2 = (H_2O_2(\text{loss}) \times 100)/SO_4^{2-}(\text{volc})$$

Thus, knowing the SO_2 proportion and assuming a reasonable travel time for SO_2 from the volcanic point source[25,26], we have estimated global SO_2 conversion rates for each of the events as conversion rate $(\%\,d^{-1}) = 1 - (\%SO_2/100)^{1/n}$ where n is the travel time in days. Calculated values of %SO_2 and SO_2 conversion rates are presented in Table 1. The %SO_2 should be regarded as an averaged proportion of SO_2 in the volcanic cloud over Greenland during the time of deposition, which is 4-12 months, depending on the event. Chemical lifetimes can be derived from the calculated conversion rates using the following equation: lifetime $(d) = [\ln(1/e)]/[\ln(1-k)]$ where k is the conversion rate ($\%\,d^{-1}$) (Table 1).

In the case of the high-latitude eruptions, the volcanic sulphur compounds were transported to the Greenland area through the upper troposphere where the SO_2 oxidation takes place primarily in the gas phase through reaction with the OH radical by the following mechanism[4]

$$OH + SO_2 + M \rightarrow HOSO_2 + M \tag{6}$$

$$HOSO_2 + O_2 \rightarrow HO_2 + SO_3 \tag{7}$$

$$SO_3 + H_2O \rightarrow H_2SO_4 \tag{8}$$

Over Greenland, the volcanic plume interacts with clouds resulting in the scavenging of SO_4^{2-} aerosol. Any SO_2 still present will react with H_2O_2. For these eruptions, our calculations indicate that 35-70% of the volcanic sulphur is still present as SO_2 by the time the plume reaches Greenland. The %SO_2 values are minimum estimates because there may have been insufficient H_2O_2 in the atmosphere to react with all of the volcanic SO_2, especially in the case of the Laki and the Katmai events where H_2O_2 concentration drops to almost zero. For these eruptions, calculated conversion rates are in the range 0.06-0.6% per day, corresponding to chemical lifetimes of the order of 2-15 days. These values are about ten times lower than those calculated near Mt Etna[27] but are very similar to those calculated for the oxidation of SO_2 in the troposphere at high latitude[28].

Sulphur compounds from the low-latitude Tambora eruption reach the high latitudes through stratospheric transport[10]. Because of the low H_2O concentration in stratospheric air, homogeneous gas-phase reactions are likely to be the main process of SO_2 oxidation[29]. McKeen *et al.*[29] proposed a stratospheric oxidation mechanism similar to the previously discussed tropospheric gas-phase reaction pathway[4]. Our data indicate that 15% of the volcanic sulphur reinjected into the troposphere over the Arctic is still present as SO_2. For the Tambora event, the SO_2 oxidation rate is of the order of 0.005-0.01% per day, which is considerably lower than rates calculated for the high-latitude events. This difference between SO_2 oxidation rates in the stratosphere and the troposphere is probably due to differences in the concentrations of OH, H_2O and O_2 or N_2, which are common third molecules for reaction (6).

These low conversion rates are consistent with the SO_2 stratospheric chemical lifetime of 100-200 days observed after the 1982 El Chichon eruption[30] and the 1974 Vulcan de Fuego eruption[31]. The SO_2 chemical lifetime in the stratosphere during non-volcanic periods, however, is of the order of one month[2]. Volcanic SO_2 injections therefore result in an increase in the SO_2 lifetime by a factor of 3-7. This difference between volcanic and non-volcanic periods cannot be explained by a direct decrease in the OH concentration owing to reaction (6) because the OH radical is recycled through the following reactions[29]

$$HO_2 + NO \rightarrow NO_2 + OH \tag{9}$$

But large SO_2 injections can lead to a lower O_3 photolysis by increasing the SO_2 opacity in the 300-nm wavelength region[2], thus indirectly affecting the OH production rate. Such a long chemical lifetime supports the hypothesis[2] of a negative feedback in volcanic-induced climate perturbation, by limiting the amount of particulate H_2SO_4 present in the stratosphere at a given time.

Oxidation of volcanic SO_2 seems to be a very slow process in the stratosphere and the SO_2 chemical lifetime is increased to several months after volcanic eruptions. The volcanic SO_2 could then be oxidized far away from the eruption site and may produce a large depletion of the H_2O_2 reservoir when this material is reinjected into the troposphere.

This peroxide depletion seems to be taking place at least on a regional scale following both high-latitude and low-latitude volcanic events. Because of the very high solubility of peroxide in water, it is very likely that this depletion is parallelled by a depletion of gas-phase peroxide. Considering that the HO_2 radical is the main source of H_2O_2 in the tropospheres[5], this may also affect the concentration or the lifetime of this reactive compound. Because of the importance of the HO_2 radical in ozone chemistry, volcanic eruptions are then likely to produce a short-term variation in both nitrogen and odd-oxygen chemistry[7]. More work will be necessary to quantify the impact on other chemical cycles in the atmosphere. ☐

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The sensitivity of terrestrial carbon storage to climate change

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THE high correlation found between atmospheric CO_2 concentration and temperature during the past 160,000 years[1,2] implicates CO_2 as an important driving force behind changes in palaeoclimate. Changes in biological processes and circulation patterns in the oceans are believed to drive glacial-to-interglacial changes in CO_2 and thus in climate. Here we estimate the role of the terrestrial biosphere in controlling atmospheric CO_2 levels during climate perturbations. By considering the coupling between vegetation distributions and climate, we perform simulations to calculate the global geographical distribution of vegetation during the last glacial maximum, 18 kyr ago. The known changes in sea level at this time, together with the simulated climate-driven spatial arrangement of vegetation, result in a mass transfer of carbon from the terrestrial biosphere to the atmosphere ranging from 30×10^9 tonnes (30 Gt, corresponding to 15 p.p.m. CO_2) to -50 Gt (25 p.p.m.). Thus, although the biosphere may have contributed to the decrease in atmospheric CO_2 of 80 p.p.m. known to have occurred at 18 kyr (refs 1, 2), it does not seem to have been a dominant factor. For simulations run with twice the present-day CO_2 levels, strong negative feedbacks appear which remove 235 Gt of carbon (128 p.p.m. CO_2) from the atmosphere.

Bioclimate schemes relate the distribution of climates and the distribution of vegetation types, classifying climates according to their thermal and moisture attributes. The climate classes are named for vegetation types with which they have been associated. This association of vegetation and climate permits the simulation of vegetation distributions for different climates.

In an earlier study[3], the contemporary distribution of global vegetation was simulated by using a refined version of the Holdridge Life Zone Scheme[4] to classify observed 30-year mean annual temperature and precipitation[5]. The climate classes were grouped and named for the vegetation types with which they have greatest geographic coincidence[6]. Unlike earlier bioclimate predictions[7-10], resulting climate classes were named for present-day vegetation types defined by the internationally recognized UNESCO classification scheme[11] rather than for the independent and generalized vegetation groups used by the bioclimate schemes. Quantitative evaluation of the simulated distribution is thus possible because a consistent vegetation terminology is used for both observed and simulated vegetation. At 1°×1° resolution for the globe, 77% of the simulated landscape, classified as 29 vegetation types, spatially corresponded with observed vegetation. The misclassifications over 23% of the land surface generally involve climatically similar vegetation types which also have similar carbon attributes.

For the study reported here, the 29 vegetation types are combined into 14 types to reflect more generalized units of carbon storage. We apply distribution relationships developed between the 14 observed UNESCO types and the observed climatology to perturbed climates. As well as today's climates, climates at 18 kyr BP (before present) and climates for doubled CO_2 levels ($2\times CO_2$) have been simulated using the general circulation model (GCM) at the Goddard Institute for Space Studies[12]. Temperature and precipitation, simulated at a 4°×5° resolution, are linearly interpolated to $2\frac{1}{2}$°. Both the $2\frac{1}{2}$° observed and simulated distributions are then regridded to 1° without further interpolation beyond that necessary for the $\frac{1}{2}$° registration. Annual temperature and precipitation used here are calculated for each 1° gridbox by adding the difference between simulated perturbed and simulated present climates to observed 30-year mean climatologies[5]. In this way, the interpretation of bioclimate patterns is rooted in observed climatology. Land area changes as 1°-gridded surface areas emerge or submerge with altered sea level. The sea level at 18 kyr is lowered by 130 and 100 m to bracket a best estimate of 121 m (ref. 13), and the sea level for the $2\times CO_2$ simulation is raised by 2 m, a likely upper estimate[14].

To evaluate the possible impact of the perturbed biosphere on the carbon cycle, we calculate the sizes of the carbon reser-

TABLE 1 Carbon densities and areal extents for 14 vegetation types

Vegetation type	Biomass ($kg\,C\,m^{-2}$)		Soil carbon ($kg\,C\,m^{-2}$)		Present area ($10^{12}\,m^2$)	18 kyr BP (−130 m) (% change)	18 kyr BP (−100 m) (% change)	$2\times CO_2$ (+2 m) (% change)
	ref. 16	ref. 15	ref. 19*	refs 17, 18				
1	15	18	10	10	19	20	17	75
2	5	5	7	8	6	−23	−31	−42
3	3	2	6	12	5	−40	−42	35
4	1	0	6	7	30	2	0	−22
5	0	0	3	1	14	−91	−91	−62
6	3	1	11	20	2	−7	−8	−17
7	3	3	8	29	2	78	76	4
8	10	11	10	10	12	32	31	40
9	9	9	14	18	16	−16	−18	5
10	6	6	12	18	6	−21	−22	−66
11	1	0	17	13	7	2	1	−63
12	0	0	0	0	3	1,223	1,171	0
13	8	13	8	11	2	170	163	−31
14	6	11	7	8	9	−89	−91	21
Global (Gt C)	748	834	1,143	1,313	132	$152\times10^{12}\,m^2$	$148\times10^{12}\,m^2$	$132\times10^{12}\,m^2$

The 14 simulated types are listed in column 1. They are: (1) tropical rain forest, (2) drought-deciduous woodland, (3) savanna, (4) arid grassland and shrubland, (5) desert, (6) mesic grassland, (7) Mediterranean forest and woodland, (8) cold-deciduous broad-leaved forest and woodland, (9) cold-deciduous needle-leaved forest and woodland, (10) evergreen needle-leaved forest and woodland, (11) tundra, (12) polar desert and ice, (13) temperate evergreen seasonal broad-leaved forest, (14) drought-deciduous and drought-seasonal broad-leaved forest. Carbon densities (columns 2–5) are based on published figures. They are constant for all climates. Areal extents of the 14 vegetation types (column 6) were bioclimatically simulated using observed climatology[4]. Simulated changes in areal extents (columns 7–9) are expressed as a percentage of present-day area (excluding Antarctica).

* Soil carbon is estimated directly from climate and averaged over the area of each vegetation type simulated from observed climate.

voirs in vegetation and soils at 18 kyr, for $2\times CO_2$ and for the present climate. Biomass estimates for vegetation types are taken from Whittaker and Likens[15] as well as from Olson *et al.*[16]. Soil carbon densities for their associated soils types are obtained by combining estimates of Ajtay *et al.*[17] and Schlesinger[18]. These carbon densities are geographic extrapolations of field measurements. The distribution of soil carbon densities is also estimated directly from climate using Post *et al.*'s superposition of isolines of soil carbon densities on the Holdridge climate space[19].

Over 75% and 60% of the present vegetated landscape was altered by the 18 kyr BP and $2\times CO_2$ simulated climates respectively. During the 18 kyr BP simulation, land temperatures dropped ~4.4 °C in the Southern Hemisphere and 8.5 °C in the Northern Hemisphere. Although the global landscape was more arid, eastern and southern coasts as well as present arid regions were wetter than today. The 100- and 130-m drop in sea level increased the land area by 12% and 15%. Regions of extreme climates (deserts, tropical rainforests and ice) show greatest sensitivity to climate change.

In this study, the biospheric carbon changes only because the distribution of vegetation types changes. The dynamics involved in both the change in the distribution of vegetation and changes in carbon density in response to changing climate are not considered. This analysis is a comparison of static snapshots of potential vegetation existing for today's, for the 18 kyr BP and for a $2\times CO_2$, climate.

Table 1 lists carbon densities for the 14 vegetation types (columns 2–5) as well as their present land areas (column 6) and percentage change in their areas associated with perturbed climates. (Numbers in the discussion refer to Olson *et al.*[16] and Post *et al.*[19] unless otherwise stated.) During the 18 kyr BP (−130 m) simulation, the advance of ice sheets claimed $25\times10^{12}\,m^2$ of present land, significantly reducing the extent of boreal vegetation. The areas of needle-leaved boreal forests (types 9 and 10) were reduced by 17% despite a 5% expansion on newly emerged land. The descent of the ice sheets and other effects of unfavourable climate as well as sea-level changes reduce the carbon stored in the 18 kyr BP boreal forests (type 9 and 10) by 80 Gt (30 Gt as biomass carbon and 50 Gt as soil carbon).

During the 18 kyr BP simulation, a large conversion of carbon occurred in the present land area of the equatorial region and in the Southern Hemisphere. Temperate and tropical broad-leaved forests (types 7, 8, 1, and 13) expanded into the cooler and wetter subtropical regions displacing subtropical broad-leaved forests and savannas (types 2, 14 and 3) which exist in climates having more strongly seasonal precipitation. The areal expansion of the less drought-seasonal forests (types 7, 8, 1 and 13) on existing and new land increases their carbon storage by 256 Gt, which is about equally divided between biomass and soil and also equally divided between new and existing land. The areas of the more drought-seasonal vegetation (types 2, 14 and 6) were reduced by 57% shrinking their carbon pool by 136 Gt (57 Gt carbon in biomass and 79 Gt as soil carbon).

Because of increased precipitation in arid regions during the 18 kyr BP simulations the area of deserts is reduced by 91%. Although 18 kyr BP desert decreases by $12\times10^{12}\,m^2$, this large area reduction represents only a 45 Gt decrease in the desert carbon pool—almost entirely as soil carbon.

Although the simulated biosphere responds dramatically to 18 kyr BP changes in climate, large regional changes in carbon reservoirs and changes in soil and biomass carbon offset one another in the global carbon budget. Global estimates of changes in carbon reservoirs from present to perturbed climates are listed in Table 2. Estimates of carbon-reservoir changes in Table 2 are broken into those effected by changes in both climate and sea level and those associated with changes in climate only. For 18 kyr BP climate with a 130 m drop in sea level, the 6% (43 Gt) gain in biomass carbon is offset by the 4% (43 Gt) decline in the soil carbon pool. Net change for the amount of carbon held in the 18 kyr BP (−130 m) biosphere is approximately zero. Like the changes in the biomass and soil reservoir, the changes in the global carbon reservoir associated with climate are also compensated by the effects of sea-level change on the biosphere. The direct effects of unfavourable 18 kyr BP climate reduce biomass carbon by 7% and soil carbon by 13%. This 200-Gt reduction of terrestrial biospheric carbon is offset by the development of vegetation and soil on new land. The $20\times10^{12}\,m^2$ of newly emerged land (Table 1) increases the carbon in the present terrestrial biomass by 13% and soil carbon by 9%. The amount and location of emerged land can account for a large increase in biospheric carbon. In this experiment, the biospheric

TABLE 2 Global terrestrial carbon reservoir changes (%) relative to today

Climate	Reservoir	Present-day carbon reservoir (Gt C)	Percent change in carbon reservoir: change in sea level (m) −130	−100	0*	+2
18 kyr BP	biomass					
	ref. 16	748	6	3	−7	—
	ref. 15	834	5	2	−8	—
	soil carbon					
	ref. 19	1,143	−4	−6	−13	—
	refs 17, 18	1,313	−1	−3	−10	—
$2\times CO_2$	biomass					
	ref. 16	748	—	—	31	31
	ref. 15	834	—	—	35	35
	soil carbon					
	ref. 19	1,143	—	—	1	1
	refs 17, 18	1,313	—	—	3	3

* Reservoir changes brought about by direct climate effects only

carbon supported on new land is as great as the changes in the biospheric carbon resulting directly from climate.

Similar changes in biospheric carbon reservoirs occurred in the 18 kyr BP (−100 m) simulation but the changes were affected by the smaller increase in land area associated with the 100 m drop in sea level. Biomass carbon storage increases by 3% (25 Gt) relative to the present reservoir whereas soils lose 6% (65 Gt). The terrestrial biosphere in this experiment loses 40 Gt of carbon.

The land climate for the $2\times CO_2$ simulation is an average of 5.4 °C warmer and is 17.5 mm d^{-1} wetter than present climate. Areas of tropical rainforests increase by 75% (Table 1) increasing their biomass and soil carbon reservoirs by 215 Gt and 130 Gt of carbon respectively. Desert (type 5) and semi-desert (type 4) regions are reduced by 60% and 20%, reducing the carbon stored in arid lands by 75 Gt. Cold-deciduous broad-leaved (type 8) forests expand northward in the Northern Hemisphere in the warmer, wetter $2\times CO_2$ climate adding 50 Gt carbon to their soil reservoirs and 60 Gt to their biomass. Areal decrease of boreal evergreen needleleaved forests (type 10) and tundra (type 11) reduces their soil carbon pool by 145 Gt.

The global terrestrial biosphere responds to the $2\times CO_2$ climate with a 31% increase in biomass a 1% increase in soil carbon. This 235-Gt carbon expansion of the terrestrial biosphere is due entirely to the change in climate (the sea level rises 2 m).

Although the spatial rearrangement of vegetation in response to perturbed climates is significant, the magnitude and direction of the net change in carbon storage remain unclear. Many caveats must accompany the resulting global carbon budgets. (1) At present, only 77% of the observed vegetated landscape can be replicated because we do not know how to express climate to define best the structural/functional attributes of vegetation. In addition, the secondary effects of soils, topography, humans and other environmental factors on the vegetation distribution are not accounted for in this study. Because misclassification of vegetation most often occurs between climatically similar vegetation types having similar carbon-storage attributes, the study's results are not highly sensitive to small errors in classification. Results are most sensitive to misclassifications in areas having a strong gradient in carbon density (for example, along a north-south transect of soil through ice, tundra and boreal forests). (2) We assume relationships now existing between climate classes and vegetation types hold for perturbed climates. In fact vegetation types existing under perturbed climates may differ in structure and function from those existing today. We also assume that vegetation is in equilibrium with climate — a condition which is never entirely met in a constantly changing climate. The assumption of steady state becomes less correct for the more slowly changing soil-carbon reservoir over what is believed to be the relatively short time period of an approaching $2\times CO_2$ climate. (3) The estimation and extrapolation of carbon densities for the geographically extensive and diverse vegetation types requires more field measurements than are presently available. Sensitivity of global terrestrial carbon storage to small errors in the carbon-storage description (Table 1) increases with the area of the vegetation type. Such errors are likely to increase with the density of carbon storage, with increasing percentage of storage in the soil (where carbon density is more difficult to measure), with diversity of carbon storage within vegetation type (making extrapolation of field measurements difficult) and inversely with the frequency of the field sampling of carbon density. The discrepancies between the minimum terrestrial carbon storage[16,19] shown in Table 2 and the maximum values[15,17,18] for a single-climate scenario are generally more than double the storage differences between climate scenarios. (4) Uncertainties and errors in GCM simulations, especially of precipitation affect the distribution of vegetation and terrestrial carbon. (5) At the GCM level of generalization and structural homogenization of vegetation types, issues of CO_2 fertilization and the functional differences of C_3 and C_4 plants cannot be addressed. (6) The 100-m and 130-m drops in sea level in the 18 kyr BP simulations are responsible for a 40-Gt difference in the amount of carbon stored in the biosphere. (7) Estimates of the changes in the net global biospheric carbon are obtained by summing large numbers often having opposite signs. Because there is a large margin of error in each component number, an accurate net change in the global terrestrial biosphere is difficult to obtain. Some generalizations, however, are possible.

In our study, both biomass and soil carbon pools during the 18 kyr BP (−130 m) simulation decreased by 200 Gt carbon on the present land area, or ~10% of the total present land reservoir. The decrease is countered by the substantial increase of carbon stored on the newly emerged land. The magnitude of this compensation is difficult to ascertain because of the uncertainties in the sea-level change and lack of understanding of the biosphere on the new landscape. By assuming a sea-level drop of 130 m and applying today's terrestrial carbon relationships, we obtain an increase in carbon storage of 200 Gt on new land, exactly compensating for the loss owing to unfavourable climate. Although our best estimate of the mass transfer of carbon between the biosphere and the atmosphere for the 18 kyr BP simulation is approximately zero, Tables 1 and 2 show possible biospheric changes ranging from an increase of 30 Gt carbon for −130 m (refs 16–18) to a decrease of 50 Gt carbon for the −100-m experiment[15,19]. Although biospheric changes do not dominate the 82-p.p.m. (165 Gt carbon) difference in atmospheric CO_2 between the Holocene and 18 kyr BP found in ice cores, their contribution may not be negligible because of uncertainties in calculating the components of large compensating factors in the biosphere–atmosphere carbon flux.

In our $2\times CO_2$ simulation, the change in sea level (+2 m) has a negligible effect on global land area. In this experiment, a terrestrial biosphere in equilibrium with a $2\times CO_2$ climate has an increased carbon storage ranging from 338 Gt (refs 15, 17, 18) to 245 Gt (refs 16, 19), or 169 p.p.m. to 122 p.p.m. This represents a potentially strong negative feedback on the atmospheric CO_2 increase.

In both cold and warm climates, the redistribution of vegetation results in carbon transfers from soil to biomass and from boreal to tropical regions. The present climate seems to be unique in its capacity to support such extensive carbon-rich boreal soils and such extensive carbon-poor arid lands.

These sensitivity experiments explore the influence the terrestrial biosphere might have had in the glacial–interglacial changes of atmospheric CO_2 as well as the possible carbon-storage role in a $2\times CO_2$ climate. We hope that this research can provide a framework to analyse and extrapolate terrestrial response to global climate changes. □

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Temperature measurements during initiation and growth of a black smoker chimney

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BLACK smoker chimneys are hollow spires formed by mineral deposition at sea-floor hydrothermal vent sites. They grow in two stages: formation of a sulphate-dominated wall (stage I), followed by precipitation of sulphide minerals on the inner side, and in pore spaces, of the wall (stage II)[1,2]. Here we present the results of an *in situ* 46-day experiment which allows direct observation of stage I growth processes by monitoring wall and fluid temperatures. The position and thickness of stage I chimney walls can change on short timescales, and are controlled by the dynamics of fluid flow both during[3] and after initial wall emplacement. The temperature in the main flow remained stable at 353±2 °C throughout the experiment, including the period during which the chimney wall was constructed. Six thermocouples, however, recorded maximum temperatures between 365 and 405 °C, which are significantly higher than most exit temperatures documented previously[4]. These latter observations bear on the question of the maximum temperatures attainable in sea-floor hydrothermal systems (refs 4–9, J. R. Delaney, manuscript in preparation).

In July 1988 an instrument equipped with an array of thermocouples housed within a test collar was deployed in the Endeavour hydrothermal vent field (47° 57′ N) on the Juan de Fuca Ridge[10,11]. The collar (Fig. 1*a*) was set over the opening of a black smoker vent and remained in place for 46 days. Temperatures (Fig. 2) were recorded every 2 min by 10 thermocouples inside the collar, 2 thermocouples outside the collar, and a platinum resistance temperature device (RTD) on the instrument platform, which was moored 1 m from the collar. The presence of the collar and thermocouples probably influenced fluid flow and mineral deposition to some extent, but some disturbance is unavoidable.

During the first 52 hours of the experiment, before chimney growth, the thermal structure of the hydrothermal plume was documented. Temperatures at TC02 (thermocouple 2), TC03 and TC09 increased rapidly to, and remained stable at, 353± 2 °C. Temperatures increased more slowly to maxima of 353± 2 °C at TC05, TC06 and TC11, 365 °C at TC08, 375 °C at TC10, 381 °C at TC07, and 405 °C at TC04. The three highest temperatures are on the boiling curve (375 °C) or in the two-phase region for a seawater-salinity fluid at 220 bars[6,12]. Excursions to ≥375 °C lasted for 90 min at TC04, 42 min at TC07 and 4 min at TC10, and were generally coincident but not simultaneous. In 1984 temperatures of 370–>400 °C and of >400 °C were measured at this site (Endeavour hydrothermal field; refs 6, 7, J. R. Delaney, manuscript in preparation) but their reliability has been disputed because these values were not reproduced on subsequent visits (temperatures were lower, but the same individual vents were not occupied) (ref. 4, J. R. Delaney, manuscript in preparation). In 1988 a temperature of 375 °C was measured 2 km to the north (ref. 5, J. R. Delaney, manuscript in preparation). Nearly all other published exit temperatures are <350±5 °C (ref. 4), including the temperature of the main flow measured in this experiment. Experimental reproduction of hydrothermal fluid compositions, however, has only been accomplished at temperatures >385 °C (refs 8, 9). Our high-temperature measurements seem to be reliable because they were all made adjacent to one another (Fig. 1*a*), and all thermocouples recorded temperatures within 2–4 °C of one another (within the error limits, ±2 °C) during deployment and recovery, and seem to have performed consistently throughout all other portions of the experiment. On the other hand, thermocouple TC02, only 4 cm from TC04, recorded a stable temperature of 353±2 °C for the duration of the experiment, including the period during which TC04 recorded 405 °C, and the thermocouples that measured anomalously high values did not record stable temperatures or temperatures similar to one another (Fig. 1*b*). The simplest explanation that accounts for all measurements is that there were two sources, one that was very stable at 353 °C, and a second, hotter source which was responsible for the >355 °C temperatures (J. R. Delaney, manuscript in preparation).

TABLE 1 Pressure difference across chimney wall

Diameter (m)	Volume flow rate ($m^3 s^{-1}$)	Pressure difference (Pa)
0.016	0.00039	0
	0.00069	6,700
0.02	0.00039	−2,200
	0.00069	0
0.025	0.00039	−2,900
	0.00069	−2,200

Pressure differences calculated using equation (2), using $y=1$ m and $f=0.04$ (ref. 3); densities for 2 °C sea water and 350 °C hydrothermal fluid are 1,003 and 674 kg m^{-3} (ref. 12), respectively.

The anomalously high temperatures were recorded only during a short time following emplacement of the collar. Within 7 hours, temperatures at TC04, TC07, TC08 and TC10 became relatively stable at 369, 361, 358 and 357 °C respectively, and drifted to 363, 356, 354 and 354 °C over the next 45 hours. During this time all other thermocouples in the collar measured stable temperatures of 353±2 °C (Fig. 1*c*, Fig. 2*a*). The initial rapid increases to 353±2 °C at TC02, TC03 and TC09 indicate that hot fluid was directed against one side of the test collar (Fig. 1). Progressive increases in temperatures at the other thermocouples to >350 °C probably reflect decreases in leakage of sea water into the collar as mineral precipitation at the collar base sealed openings.

During the third day of the experiment, temperatures at all thermocouples except TC02 and TC03 began to change rapidly (Fig. 2*b*), indicating the sudden onset of seawater entrainment over the top of the collar (Fig. 1*d*). The cause of entrainment is unclear but possibilities include development of an instability in the turbulent plume, or a sudden decrease in flow rate. The data show that once sea water was entrained, a barrier formed rapidly (in 6–12 h; Fig. 2*b*), isolating TC06, TC07 and TC11 from the fluid. Over the following 3.5 days, temperatures at TC06, TC07, TC10 and TC11 decreased to <106 °C, and temperatures at TC05, TC04, TC09 and TC08 fluctuated rapidly, consistent with being in zones of mixing. TC03 also showed minor fluctuations, at one point recording a temperature of 372 °C; TC05 recorded 372 °C 30 min before the TC03 measurement. Throughout this time, and during wall emplacement, TC02 remained stable at 353 ± 2 °C. After 3.5 days, temperatures at each thermocouple became relatively constant. TC08 recorded a temperature of 309 °C, consistent with being located in the newly formed chimney wall; TC09 and TC04 recorded temperatures of 342 and 343 °C, indicating that they were in or against the inner side of the chimney wall, and TC05 and TC03 recorded stable temperatures of 353 ± 2 °C, indicative of being in rapidly moving high-temperature fluid.

During the remaining 40 days of the experiment temperatures at TC06, TC07, TC10 and TC11 decreased to ambient temperatures (Fig. 2*a*) as the wall became impermeable to leakage of hydrothermal fluid. Temperatures at TC02 and TC03 remained stable at 353 ± 2 °C, and temperatures at TC04, TC08, TC05 and TC09 decreased to 21, 67, 134 and 283 °C, respectively. On recovery, TC04 was outside the wall, TC08 was against the outer wall, and TC05 and TC09 were embedded in the wall (Fig. 1*h*). The final locations of these thermocouples and the recorded decreases in temperature with time are most easily accounted for by inward thickening of the wall coupled with

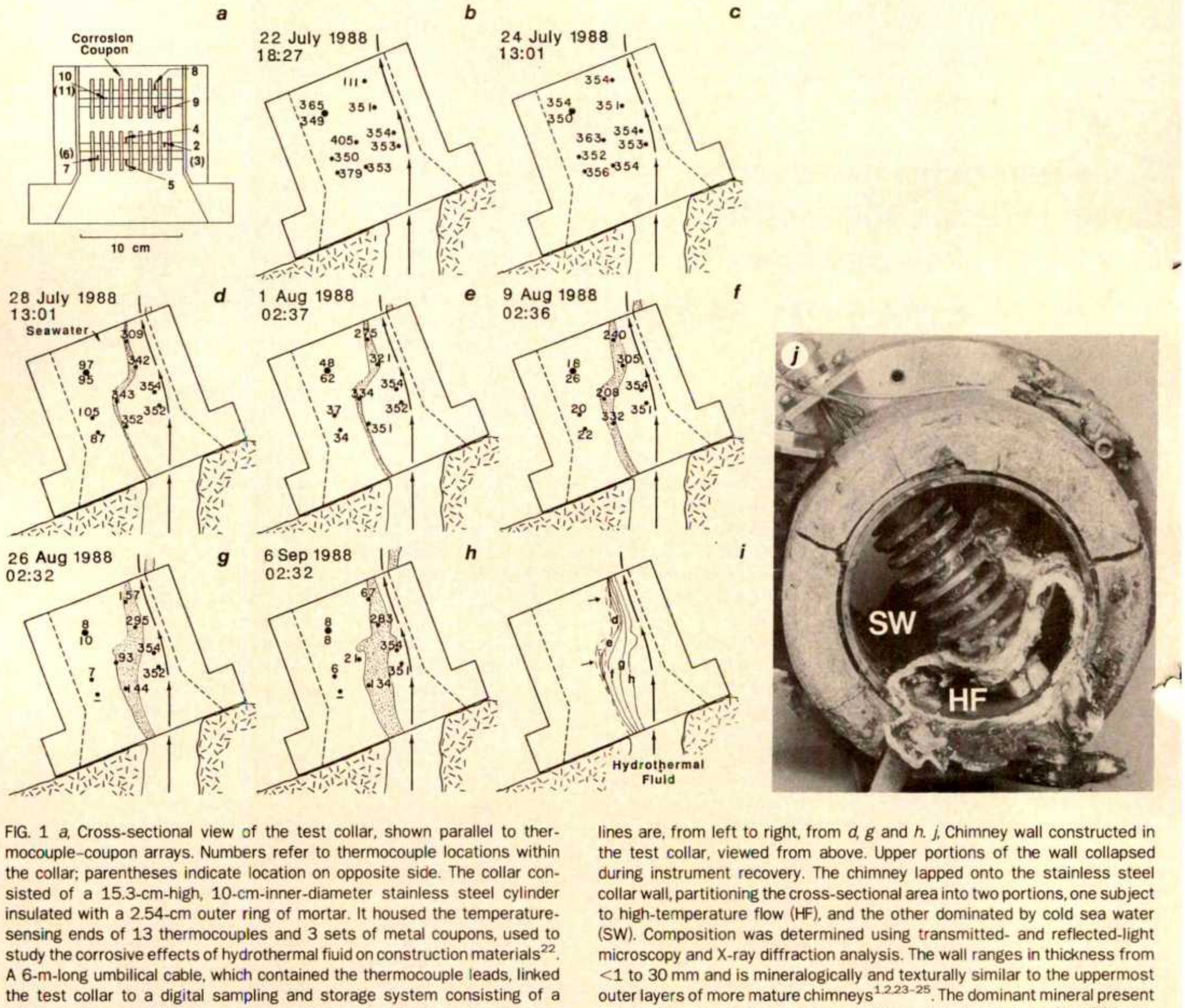

FIG. 1 *a*, Cross-sectional view of the test collar, shown parallel to thermocouple–coupon arrays. Numbers refer to thermocouple locations within the collar; parentheses indicate location on opposite side. The collar consisted of a 15.3-cm-high, 10-cm-inner-diameter stainless steel cylinder insulated with a 2.54-cm outer ring of mortar. It housed the temperature-sensing ends of 13 thermocouples and 3 sets of metal coupons, used to study the corrosive effects of hydrothermal fluid on construction materials[22]. A 6-m-long umbilical cable, which contained the thermocouple leads, linked the test collar to a digital sampling and storage system consisting of a microprocessor, solid-state memory and battery pack. Corrosion coupons exposed to fluids included nickel and titanium alloys, and stainless steel. Only stainless steel coupons exhibited corrosion. *b*–*g*, Cross-sectional view of collar showing measured temperatures and inferred wall positions (stippled) during the experiment. The 20° angle of the test collar from vertical represents the inferred orientation during the experiment, based on observations of flow during deployment, and on the 20° angle of the uppermost chimney wall to the test collar on recovery. *h*, Temperatures recorded before instrument recovery, and location of the wall inside the collar on recovery. *i*, Location of the inner (solid lines) and outer (dashed lines) chimney wall as a function of time; wall positions are taken from *d*–*h*, and are labelled as such. Arrows indicate places on outer wall subject to dissolution. Dashed lines are, from left to right, from *d*, *g* and *h*. *j*, Chimney wall constructed in the test collar, viewed from above. Upper portions of the wall collapsed during instrument recovery. The chimney lapped onto the stainless steel collar wall, partitioning the cross-sectional area into two portions, one subject to high-temperature flow (HF), and the other dominated by cold sea water (SW). Composition was determined using transmitted- and reflected-light microscopy and X-ray diffraction analysis. The wall ranges in thickness from <1 to 30 mm and is mineralogically and texturally similar to the uppermost outer layers of more mature chimneys[1,2,23–25]. The dominant mineral present is anhydrite, ranging in size from <10 μm to >1 mm, averaging 500 μm. Fine grains (<5–20 μm) of pyrrhotite, pyrite, chalcopyrite and sphalerite or wurtzite intergrown with and included in anhydrite are present in small amounts (<5–10%). Minerals do not exhibit any signs of replacement. A thin (<1 mm) partially continuous layer of chalcopyrite is present on the inner side of the wall. At some locations, ridges of anhydrite project into the open channel. X-ray diffraction analyses of the thickest portion of the wall indicate the presence of caminite (magnesium hydroxysulphate hydrate). The only other reported occurrence of caminite, which was also intergrown with anhydrite, was in the outer portion of a black smoker chimney wall from 21° N on the East Pacific Rise[26–28].

some dissolution and removal of the outer wall at TC04 and TC08. Recorded temperatures and inferred wall positions for six different times are displayed in Fig. 1.

The experiment allowed the first direct observation of a very young chimney wall. The wall that grew up through the collar is composed dominantly of anhydrite (Fig. 1*j*). Only a very thin (<1 mm) layer of chalcopyrite is present against the inner side of the wall, and the few sulphide grains intergrown with and included in anhydrite do not exhibit any signs of replacement. The time-series records indicate that the wall was emplaced on a timescale of days, consistent with previous observations[2,13], and that it then thickened inward by as much as 2.5 cm over a time interval of weeks (Fig. 1*i*). The lack of replacement of earlier formed minerals, and presence of only minor amounts of chalcopyrite lining the innermost (youngest) chimney wall, indicate that stage I growth prevailed. Inward growth was dominated by anhydrite deposition, which requires the presence of sulphate, a component available in sea water but not in hydrothermal fluid[14]. If sulphate is supplied by the inward advection of sea water, then the rate of thickening is controlled by the magnitude of the pressure gradient across the wall, and by permeability K as expressed by Darcy's law

$$u = -\frac{K}{\eta}\frac{P_i - P_o}{dx} \qquad (1)$$

where u is advection rate, η is viscosity, dx is the width of the porous medium, and P_i and P_o are pressure on the inner and outer sides of the wall[15]. Inward growth by deposition of anhydrite should cease when P_i becomes equal to or greater than P_o.

The magnitude of the pressure difference required to produce the observed thickening of the wall can be estimated by considering (1) the flux of sulphate necessary to account for deposition of a 0.025-m-thick wall of anhydrite (molar volume 4.6×10^{-5} m^3 mol^{-1}; ref. 16) in 40 days, which is 1.6×10^{-4} mol m^{-2} s^{-1}, and (2) the advection rate of sulphate (concentration in sea water of 28 mol m^{-3}; ref. 17) necessary to provide this flux, 5.7×10^{-6} m s^{-1}. This advection rate could be generated by a pressure difference of −2,253 Pa across a 0.025-m-thick wall (calculated using equation (1)), assuming an average viscosity of hot seawater-salinity fluid of 5×10^{-4} Pa s (ref. 18), and a permeability similar to that of sandstone ($10^{-13.5}$ m^2; ref. 14). Conditions under which such a pressure difference would exist can be evaluated using a method that considers the fluid dynamics of high-velocity flow inside the chimney channel[3]. The pressure difference inside a chimney of constant diameter d can be calculated by summing the buoyancy force with a friction loss term

$$P_i - P_o = (\rho_i - \rho_o)gy + \frac{8f\rho_i Q^2}{\pi^2 d^5}\,y \qquad (2)$$

where ρ is fluid density, y is depth in the channel, f is a friction factor, and Q is volume flow rate[3]. Calculated pressure differences at a depth of 1 m from the top of the chimney as functions of Q and d are given in Table 1. The value of d that corresponds to a pressure difference of zero for a given constant flow rate is termed the 'stable' chimney diameter[3]. A pressure difference of the order of −2,200 Pa at a depth of 1 m in the channel, which would permit inward thickening, could result either from a decrease in volume flow rate after initial emplacement at a stable diameter[3], or from emplacement of the wall at a diameter larger than this. The larger diameter could occur if wall emplacement mimics the outer bound of the plume which expands above the chimney orifice[19]. In either case inward growth would cease when a stable diameter for the existing volume flow rate Q is achieved.

The idea of a stable diameter can also be used to explain why the chimney wall grew within the test collar instead of on top of it. Formation of a chimney wall with a stable diameter of 10 cm would require an extremely high flow rate of 26 kg s^{-1} (0.0384 m^3 s^{-1}). Estimated mass flow rates from vigorous smokers at 21° N on the East Pacific Rise are significantly less, of the order of 1.5 kg s^{-1} (ref. 19). The wall formed within the test collar had a hydraulic radius (cross-sectional area/perimeter) of 0.005 m, equivalent to a circular diameter of 0.02 m, which corresponds to a more reasonable flow rate of 0.5 kg s^{-1} (0.00069 m^3 s^{-1}). The experiment indicates that the orifice diameter and position and thickness of the anhydrite-dominated stage I wall are not just a result of initial mixing between the plume and ambient sea water[3], but are also controlled by the dynamics of fluid flow in the chimney channel after wall emplacement.

This experiment demonstrates that *in situ* monitoring of vent phenomena is possible. The extent to which the solid collar wall and coupons influence mixing and/or fluid flow in the chimney

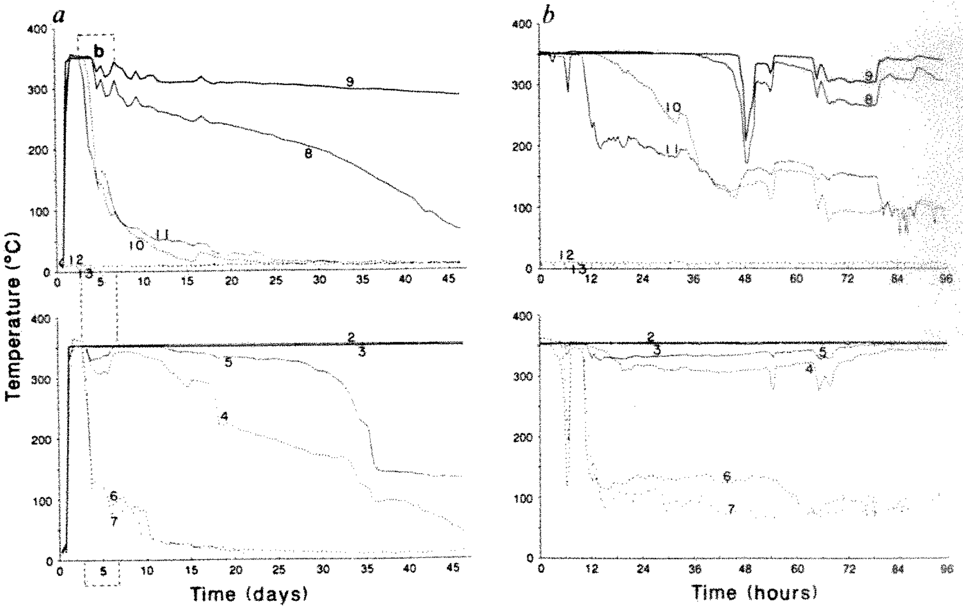

FIG. 2 *a*, Average temperatures for 12-h intervals measured over 46 days. Maximum temperatures recorded by TC03, TC04, TC05, TC07, TC08 and TC10 were 372, 405, 372, 381, 365 and 375 °C respectively. The remaining thermocouples in the test collar recorded maximum temperatures of 353 ± 2 °C, and TC02 recorded 353 ± 2 °C for the duration of the experiment. *b*, Average temperatures for 30-min intervals measured during the initial wall-building stage. Numbers refer to thermocouples. Immediately outside the test collar, temperatures varied between 6 and 16 °C for the duration of the experiment (TC12 and TC13), and at the platform containing the sampling and storage system the temperature varied from 2.5 to 3.2 °C. Temperatures were measured using ungrounded type K (chromel–alumel) thermocouples with error limits of ±2.2 °C up to 277 °C and ±0.75% for temperatures between 277 and 1,260 °C. The electronics package and reference RTD were moored 1 m away from the active vent, housed in a 1.77-cm-thick PVC case located at the end of a 6-m-long cable. Calibration before deployment consisted of placing the electrical-junction box in a thermostatically controlled freezer and the test collar and thermocouples in a vessel of boiling water. During the 24-hour test the RTD and all thermocouples performed within the expected error limits. Recalibration after the experiment was not done because it would have required dissolution of the chimney wall.

channel can be minimized in future experiments by modifying the instrument—for example, by eliminating the coupons and suspending a set of thermocouples above the collar. Longer experiments can be coupled with time-lapse camera studies[20,21] so that wall position and upward growth rates can be discerned. This technique can be used to investigate transport and chemical reaction across steep thermal and chemical gradients, the time-scale and long-term stability of sea-floor vent structures, and the stability and range of temperatures exhibited in hydrothermal systems. ☐

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Eclogitic diamonds of Proterozoic age from Cretaceous kimberlites

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DIAMONDS with eclogitic inclusions ('eclogitic diamonds') from the Premier kimberlite and Argyle lamproite were previously shown to be of Proterozoic age[1–3] and distinct in origin from peridotitic diamonds of Archaean age[4] and deep-continental-mantle (tectospheric[5]) provenance. The Premier and Argyle host rocks are themselves of Proterozoic age, suggesting a link between eclogitic diamond formation and kimberlite or lamproite magmatism[1]. Here we test this link by examining eclogitic diamonds from younger southern African kimberlites. Eclogitic garnet and clinopyroxene inclusions in Orapa and Finsch diamonds yield Sm–Nd isochron ages of 990 and 1,580 Myr respectively, compared with host kimberlite emplacement ages of ~100 Myr. These eclogitic diamond ages are again Proterozoic, and distinct from the ~3,200-Myr age of Finsch peridotitic diamonds[4]. Thus peridotitic and eclogitic diamonds from the same locality are evidently both xenocrysts, having been stored in the deep continental mantle beneath the Kaapvaal craton for extended periods before being sampled by kimberlite. Such mantle storage may not be detected by the ^{40}Ar–^{39}Ar laser-probe dating technique as applied to Premier diamonds[2,3].

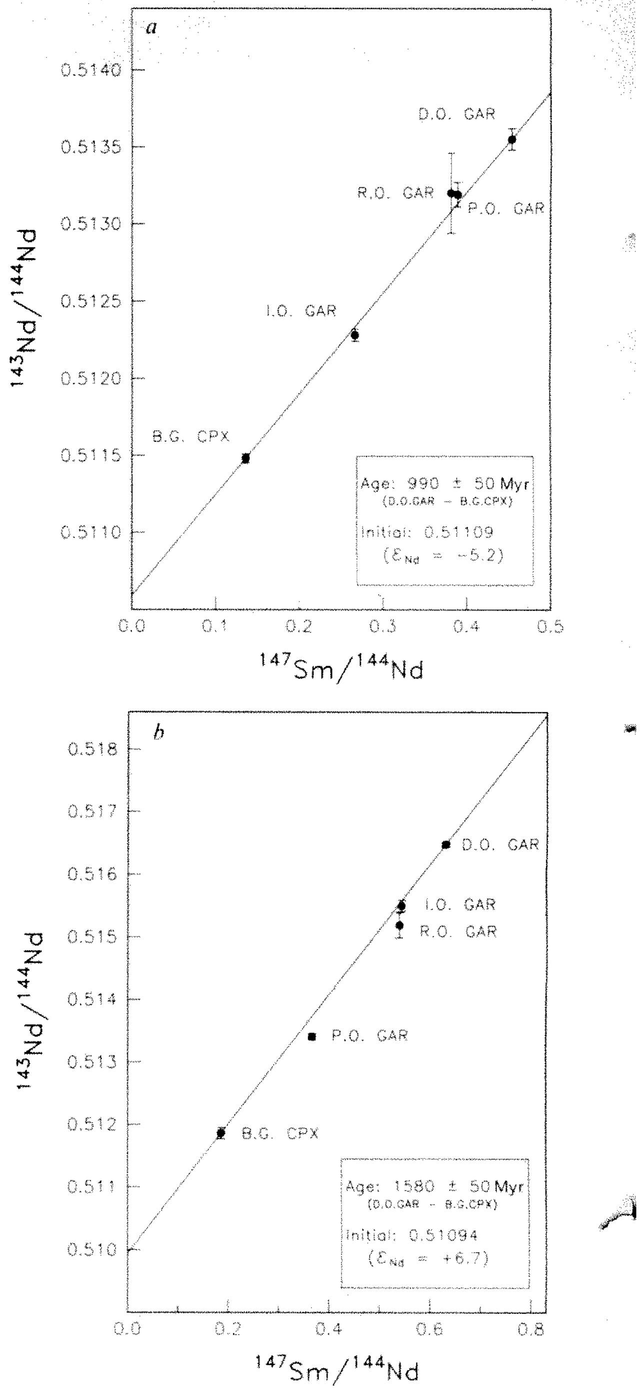

FIG. 1 Sm–Nd isochron diagrams for eclogitic garnet and clinopyroxene inclusions in Orapa (*a*) and Finsch (*b*) diamonds with sample abbreviations as in Table 1. The ages are calculated for the deep-orange garnet/bluish-green clinopyroxene pairs, using $\lambda_{Sm} = 6.54 \times 10^{-12}$ yr^{-1}, with assigned errors derived from maximum and minimum slopes allowed by $2\sigma_{mean}$ measurement errors.

The Premier kimberlite, where the ^{40}Ar–^{39}Ar laser-probe dating technique was first tested on eclogitic diamond inclusions[2,3], is an unusual case in which similar diamond crystallization and kimberlite emplacement ages cannot be resolved by current dating techniques. Nevertheless, these diamonds have nitrogen aggregation characteristics requiring a minimum 1–10 Myr period of mantle storage (within the errors of the age determinations) and implying a xenocrystal relationship to the host kimberlite itself[1]. R. Burgess (personal communication) has recently

TABLE 1 Isotopic composition of eclogitic garnet and clinopyroxene inclusions

	Wt (mg)	Rb	Sr	$^{87}Rb/^{86}Sr$	$^{87}Sr/^{86}Sr$	$^{87}Sr/^{86}Sr_i$	Sm	Nd	$^{147}Sm/^{144}Nd$	$^{143}Nd/^{144}Nd$	$^{143}Nd/^{144}Nd_i$
Orapa*											
d.o. gar	3.04	0.002	3.49	0.0020	0.70819 (2)	0.70816	2.129	2.840	0.4533	0.51405 (7)	0.51109
i.o. gar	2.17	0.009	7.02	0.0036	0.70775 (3)	0.70770	2.328	5.273	0.2669	0.51278 (4)	0.51104
p.o. gar	0.59	0.004	4.89	0.0022	0.70812 (4)	0.70809	1.671	2.595	0.3893	0.51369 (8)	0.51115
r.o. gar	0.71	0.012	2.23	0.016	0.71068 (5)	0.71045	2.393	3.793	0.3815	0.51370 (26)	0.51121
b.g. cpx	1.78	0.203	356.6	0.0017	0.70608 (3)	0.70606	1.229	5.458	0.1361	0.51198 (3)	0.51109
Finsch†											
d.o. gar	2.17	0.002	3.62	0.0015	0.70512 (5)	0.70509	1.917	1.839	0.6308	0.51748 (4)	0.51094
i.o. gar	1.11	0.005	4.09	0.0038	0.70661 (5)	0.70652	2.068	2.302	0.5435	0.51650 (9)	0.51086
p.o. gar	0.85	0.007	7.79	0.0026	0.70523 (3)	0.70517	2.148	3.551	0.3658	0.51441 (5)	0.51062
r.o. gar	0.31	0.015	1.34	0.033	0.71161 (24)	0.71086	0.723	0.811	0.539	0.51619 (20)	0.51060
b.g. cpx	0.48	0.214	111.6	0.0055	0.70474 (2)	0.70462	0.973	3.172	0.1854	0.51286 (9)	0.51094
F11‡	1.10	0.001	7.09	0.0002	0.70501 (3)	0.70501					

Concentrations in p.p.m. (μg per g), after correction for minimum applicable blanks: Rb, 3 pg; Sr, 4 pg; Sm, 0.6 pg; Nd, 1.5 pg. Estimated analytical uncertainties in concentrations (ignoring small-sample weighing errors) are indicated by the number of significant digits. Present-day $^{87}Sr/^{86}Sr$ and $^{143}Nd/^{144}Nd$ ratios are normalized to 0.70800 and 0.51264 for standards E & A $SrCO_3$ and BCR-1, respectively. In-run precision is indicated by the errors ($2\sigma_{mean}$) in the least-significant digits given in parentheses. Initial ratios (denoted by subscript i) calculated for Orapa and Finsch diamond crystallization ages of 990 and 1,580 Myr, respectively, using decay constants $\lambda_{Rb} = 1.42 \times 10^{-11}$ yr^{-1} and $\lambda_{Sm} = 6.54 \times 10^{-12}$ yr^{-1}. Inclusion-bearing diamonds were selected from the −6 +5 diamond sieve fraction (1.8–2.2 mm diameter) of general mine production.

* Orapa composites: ~160 deep-orange garnets (d.o. gar), 110 intermediate-orange garnets (i.o. gar), 60 pale-orange garnets (p.o. gar) and 80 reddish-orange garnets (r.o. gar) (average weight 16 μg); ~220 bluish-green clinopyroxenes (b.g. cpx) (average weight 8 μg).

† Finsch composites: ~150 d.o. gar, 80 i.o. gar, 130 p.o. gar and 60 r.o. gar (average weight 11 μg); ~90 b.g. cpx (average weight 5 μg).

‡ Single large d.o. gar successfully analysed for Rb–Sr only in 1985.

obtained ^{40}Ar–^{39}Ar ages for eclogitic clinopyroxene inclusions in Orapa diamonds approaching that of the host kimberlite. Whereas diamond encapsulation precludes diffusive exchange of elemental species between inclusions that are not touching, these species are able to diffuse through the inclusion minerals themselves at mantle temperatures and pressures. Further, although cations such as Sm, Nd, Rb, Sr and K remain within the crystal structures of the inclusion minerals, the noble gas Ar seems to be able to accumulate at inclusion/diamond interfaces, effectively separating radiogenic Ar from the parent K and resetting the K–Ar clock within clinopyroxene until removal to the surface. This technique may therefore give ages indicative more of the kimberlite emplacement event than of the diamond crystallization event.

The Orapa kimberlite is located in the Archaean Limpopo Mobile Belt in northeastern Botswana. A zircon U–Pb age of 93 Myr provides the most reliable estimate of emplacement age[6], which is Cretaceous and consistent with the preservation of crater facies deposits[7]. The Orapa locality was chosen for this study because of its relative abundance of diamonds containing eclogitic inclusions. The eclogitic and lesser websteritic assemblages of orange garnet, bluish-green clinopyroxene, sulphides and orthopyroxene together comprise >85% of inclusions in Orapa diamonds[8].

The Finsch kimberlite is located in the southwestern interior of the Archaean Kaapvaal craton. In the absence of zircons, a phlogopite Rb–Sr age of 118 Myr provides the most reliable estimate of emplacement age[9]. The Finsch locality was chosen because of the Archaean age of its dominant peridotitic-inclusion-bearing diamonds[4] and because of a parallel study of individual large diamonds containing eclogitic garnet inclusions, by Smith *et al.* (ref. 10 and manuscript in preparation). The eclogitic assemblage of orange garnet, bluish-green clinopyroxene and sulphides accounts for <2% of inclusions in Finsch diamonds[11], so extensive sorting was required to collect sufficient material for analysis.

Small-sample preparation techniques and mass spectrometric facilities for the determination of Sm, Nd, Rb and Sr concentrations and isotope ratios (Table 1) were the same as those used in ref. 1. Here, further categories of garnet were identified on the basis of subtle variations in colour. Major-element analyses of single specimens of each of the resultant deep-, intermediate-, pale- and reddish-orange garnet and bluish-green clinopyroxene categories are reported in Table 2. By comparison with world-wide occurrences[12], these are all clearly eclogitic with the possible exception of the Orapa reddish-orange garnet, which displays a websteritic character[8]. This colour characterization is used for convenience in separating components with a spread of Sm/Nd ratios between those of deep-orange garnet and bluish-green clinopyroxene, which constitute a cogenetic pair in rare bimineralic-inclusion-bearing diamonds (not used in this study)[1]. Otherwise, we used the same analytical strategies and precautions with respect to composites as in ref. 1. In particular, Rb/Sr ratios for both garnet and clinopyroxene are extremely low, resulting in barely significant increases in $^{87}Sr/^{86}Sr$ ratio on a 1,000-Myr timescale. Thus, $^{87}Sr/^{86}Sr$ ratios can be used to examine the cogeneticity of garnet and clinopyroxene inclusions from separate diamonds, which have been paired to derive ages from Sm–Nd isochrons.

The Orapa deep-orange garnet/clinopyroxene pair defines a two-point Sm–Nd isochron age of 990 ± 50 Myr (Fig. 1*a*), where the error is derived from the maximum and minimum slopes allowed by $2\sigma_{mean}$ measurement errors (Table 1). The intermediate-, pale- and reddish-orange garnets with intermediate Sm/Nd ratios also lie on the isochron, within the errors. Regression analysis of the complete data set is not warranted, however, because the initial $^{87}Sr/^{86}Sr$ ratios of the garnets (0.7077–0.7105) are all higher than that of the clinopyroxene (0.7061) as also shown on a present-day Sr isotope mixing diagram (Fig. 2). Ideally, the deep-orange garnet and the clinopyroxene should have the same $^{87}Sr/^{86}Sr$ ratio if they are cogenetic (as in diamonds with bimineralic inclusions[1]) but the actual datum for this composite in Fig. 2 indicates that the colour categories are unreliable and/or were not well separated during sample preparation. The reddish-orange garnet has the highest $^{87}Sr/^{86}Sr$ ratio, making it the other obvious endmember component. Nevertheless, the shape of the Orapa data field in Fig. 2 rules out simple two-component mixing as an explanation for the linear relationship in Fig. 1*a*.

Similarly, the Finsch deep-orange garnet/clinopyroxene pair defines a two-point Sm–Nd isochron age of 1,580 ± 50 Myr (Fig. 1*b*). The intermediate-, pale- and reddish-orange garnets again have intermediate Sm/Nd ratios but the latter two lie well below the isochron. The initial $^{87}Sr/^{86}Sr$ ratios of the garnets (0.7050–0.7109), including that for a single large deep-orange garnet inclusion (F11 in Table 1), are again higher than that of the clinopyroxene (0.7046) but in this case the deep-orange garnet and clinopyroxene have reasonably similar values (Fig. 2). Again, the reddish-orange garnet has the highest $^{87}Sr/^{86}Sr$ ratio. Further, a single large Finsch eclogitic garnet inclusion analysed by Smith *et al.*[10] lies on the 1,580-Myr isochron in Fig. 1*b* (at 0.702, 0.51818(5)) albeit with a slightly lower $^{87}Sr/^{86}Sr$ ratio (0.7041).

The $^{87}Sr/^{86}Sr$ ratios of the different garnet composites indicate the presence of several different components in the eclogitic inclusion assemblage at both localities. The two extremes are

TABLE 2 Major-element composition of eclogitic garnet and clinopyroxene inclusions

	Orapa					Finsch				
	d.o. gar	i.o. gar	p.o. gar	r.o. gar	b.g. cpx	d.o. gar	i.o. gar	p.o. gar	r.o. gar	b.g. cpx
SiO_2	39.49	40.41	39.80	41.01	55.40	38.45	39.42	40.93	39.01	55.80
TiO_2	0.66	0.44	0.55	0.51	0.99	0.37	0.35	0.12	0.16	0.51
Al_2O_3	22.17	23.17	22.75	21.20	9.68	21.78	22.82	22.78	21.42	9.98
Cr_2O_3	0.08	0.05	0.07	2.27	0.15	0.05	0.04	0.31	0.08	0.11
FeO	18.84	16.27	17.75	13.79	7.79	25.62	17.53	17.90	25.56	5.27
MnO	0.42	0.36	0.37	0.46	0.11	1.16	0.37	1.16	1.40	—
MgO	9.53	12.38	9.65	15.46	10.52	8.20	8.65	14.97	8.27	10.00
CaO	8.90	7.36	9.10	4.92	9.09	4.63	11.31	2.39	4.03	11.84
Na_2O	0.18	0.19	0.18	0.04	5.60	0.16	0.17	0.11	0.14	5.83
K_2O	—	—	—	—	0.37	—	—	—	—	0.07
Total	100.27	100.63	100.22	99.66	99.70	100.42	100.66	100.67	100.07	99.41

Concentrations (wt%) were determined by electron microprobe.

represented by the 'pure' deep-orange garnet/clinopyroxene pair and the reddish-orange garnet. Nevertheless, the Sm–Nd isotope data conform reasonably well to isochron relationships implying a more restricted range of initial $^{143}Nd/^{144}Nd$ ratios than the range of initial $^{87}Sr/^{86}Sr$ ratios in each case. This can be understood in terms of a trace-element-enriched (high Rb/Sr, low Sm/Nd) character for the precursor of the reddish-orange garnet, resulting in radiogenic initial $^{87}Sr/^{86}Sr$ and nonradiogenic initial $^{143}Nd/^{144}Nd$ ratios for such components. Thus, the isochron relationships in Fig. 1 seem to have age significance (rather than being mixed lines) and the corresponding initial $^{143}Nd/^{144}Nd$ ratios indicate a dominantly enriched precursor at Orapa ($\varepsilon_{Nd} = -5$; $\varepsilon_{Nd} = \{[(^{143}Nd/^{144}Nd)_{sample}/(^{143}Nd/^{144}Nd)_{bulk\ Earth}] - 1\} \times 10^4$) and a depleted precursor at Finsch ($\varepsilon_{Nd} = +7$). This difference is also reflected in the carbon isotopic composition of eclogitic diamonds which at Finsch have $\delta^{13}C$ values of −3 to −8‰ (ref. 13; $\delta^{13}C(‰) = [(^{13}C/^{12}C)_{sample}/(^{13}C/^{12}C)_{standard}] - 1$, PDB standard consistent with a depleted upper-mantle carbon source, and at Orapa values of −3 to −23‰ (P. Deines *et al.*, manuscript in preparation), which may indicate the involvement of recycled crustal carbon (M. B. Kirkley *et al.*, manuscript in preparation).

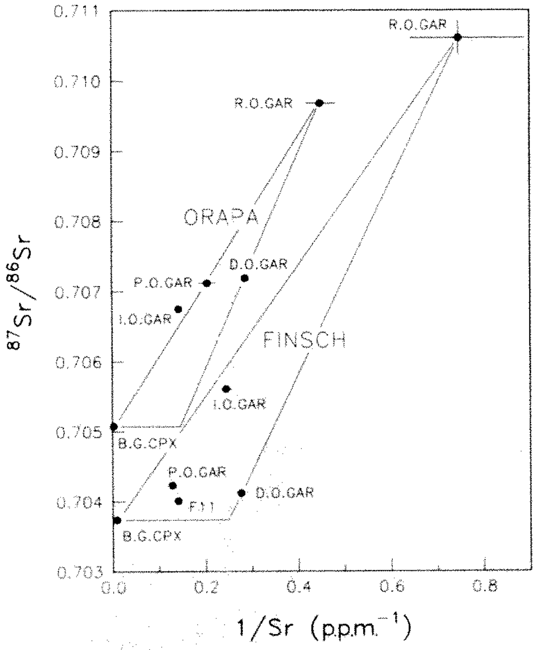

FIG. 2 Sr isotope mixing diagram with present-day $^{87}Sr/^{86}Sr$ plotted against reciprocal Sr concentration for eclogitic inclusions in Orapa and Finsch diamonds. Errors in Sr concentration are dominated by a nominal small-sample weighing error of ±0.05 mg. Mixing curves are straight lines and nominal mixing envelopes for Orapa and Finsch inclusion composites are indicated although no direct mixing of garnet and clinopyroxene is implied.

Thus there is clear evidence that eclogitic diamond formation involved at least two enriched components at Orapa and both depleted and enriched components at Finsch. Also, the precursor Nd and C isotope signatures of eclogitic diamonds from Finsch and Orapa resemble those from Premier and Argyle[1], respectively. In contrast to peridotitic diamond formation, which can be correlated with early Archaean komatiite magmatism and continental mantle stabilization[4], eclogitic diamond formation has yet to be correlated with regional tectonothermal events, although subduction is a distinct possibility. Further, the crystallization ages of peridotitic and eclogitic diamonds from Finsch differ by more than 1,500 Myr, and their precursors have contrasting enriched and depleted isotope signatures. The radiogenic Sr component evident in reddish-orange garnet inclusions in eclogitic diamonds may be derived from subducted crust and/or the enriched garnet harzburgite host assemblages of peridotitic diamonds stored in tectospheric mantle since the Archaean[4].

More generally, trace-element-depleted components (characterized by positive ε_{Nd} values) are derived from asthenospheric mantle (the source of mid-ocean-ridge basalts) whereas trace-element-enriched components (particularly those with negative ε_{Nd} values) are derived from continental tectospheric mantle and/or continental crust. As a corollary, enriched components with negative ε_{Nd} values are difficult to obtain directly from metamorphosed ocean crust. Finally, the combination of components in the above-mentioned eclogitic diamonds must be assembled and stored in stable upper mantle at depths of 150–200 km (ref. 14) on a 1,000-Myr timescale. Such eclogitic diamonds therefore most plausibly reflect local entrapment, mixing and crystallization of asthenosphere-derived magmas (which may include crustal components recycled by subduction) in tectospheric mantle beneath the Kaapvaal craton during the Proterozoic. This ancient continental-mantle keel subsequently survived convective disruption leaving original peridotitic and later eclogitic diamonds available for sampling by kimberlite in the Cretaceous. □

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Involvement of the oxygen minimum in benthic zonation on a deep seamount

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LOW oxygen concentration in the seawater column reduces the abundance of midwater consumer populations[1,2], which can enhance the supply of undegraded organic matter reaching the benthos. Low oxygen concentration in the water at the bottom can exclude most tolerant species from benthic habitats[3–5]. The interception of the seafloor with pronounced oxygen-minimum zones can produce steep gradients in benthic assemblages. We now present evidence for this interaction on Volcano 7, an oceanic seamount penetrating the oxygen-minimum zone in the eastern tropical Pacific. Submersible observations revealed only a few benthic species at the summit (730–750 m), where oxygen levels were lowest. Just tens of metres below, megafaunal and macrofaunal abundances were extremely high. Sediment organic carbon, a benthic food indicator, was unusually high. We hypothesize that a dynamic low-oxygen interface physiologically restricts benthos on the upper summit, that the enriched sediment is a result of reduced consumption and degradation of sinking material in the oxygen-minimum zone, and that this high benthic food level supports the unusually high benthic abundance. Sharp benthic zonation associated with oxygen concentrations may also be preserved in the palaeoceanographic record[4,6].

Volcano 7, an inactive seamount[7] at 13° 23′ N, 102° 27′ W, of 20-km diameter, rises from a depth of 3,400 m to 730 m. Its benthic and pelagic ecology were studied with submersible and shipboard sampling (including conductivity temperature depth (CTD) hydrocasts, water samples, box cores, photography, plankton tows, current meters and sediment traps) on a cruise in November 1988. Benthic data from June 1984 are also discussed.

The eastern tropical Pacific is characterized by a shallow mixed layer and a pronounced oxygen minimum[8]. At the Volcano 7 site, the mixed layer was 22 m deep, and the base of the euphotic zone was at 85 m. Sharp peaks of fluorescence, particles, chlorophyll *a*, phytoplankton, protozoans and zooplankton occurred just below the mixed layer (Fig. 1). The core of the oxygen minimum zone (oxygen <0.5 ml per litre of seawater) occurred from median depths of 72–1302 m, and the lowest oxygen (≤0.1 ml per litre) from median depths of 288–1,077 m (Fig. 1*a*). Thus the base of the euphotic zone was within the oxygen-minimum zone, and particles sinking out of the euphotic zone immediately entered a broad region of low oxygen and reduced plankton abundance.

There is evidence that undegraded surface-derived material reached the seamount summit. Bottom-moored particle interceptor traps[9,10] collected intact microplanktonic organisms common in surface waters. Excess Pb-210 activity profiles[11] indicated high sedimentation rates on the summit of 0.3–0.7 mm yr^{-1}. Sediment organic carbon (up to 3.9%), organic nitrogen (up to 0.55%), chlorophyll *a* (up to 25 μg g^{-1}) and sediment bacterial abundances were remarkably high on the summit, especially at depths <770 m (Fig. 2). Here, C/N ratios were near 7.0 (Fig. 2*c*), indicating the presence of fresh organic matter[12]. Green flocculent material was present on the bottom in both November 1988 and June 1984. The supply of undegraded organic material to the benthos may be more continuous in this region of reduced seasonality[13] than in higher latitudes, where benthic fluxes are strongly seasonal[14,15].

Strong megafaunal zonation was observed on the summit of the seamount (Figs 3 and 4). The uppermost summit (<750 m) was visibly depauperate (Fig. 3*a* and *b*). Gradual increases in megafauna occurred in a transition zone from 750–770 m.

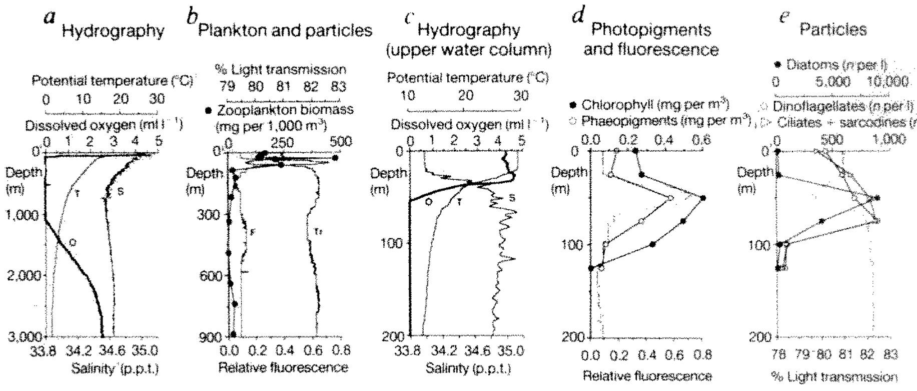

FIG. 1 Vertical profiles through the water column of physical, biological and chemical parameters. The sharp shallow thermocline is also an abrupt transition from oxygenated mixed-layer water to the very low-oxygen water in the oxygen-minimum zone. Concentrations of organisms (day and night), photopigments and particles peak sharply within the thermocline, then drop abruptly to low levels in the oxygen-minimum zone. Temperature, salinity and dissolved oxygen in the water column were measured with a SBE 19 Seacat CTD Profiler. Dissolved oxygen concentrations were calibrated by the Winkler titration method[26] on individual water samples from hydrocasts, and the oxygen values from the CTD electrode were transformed on the basis of a linear equation relating the two sets of measurements. Thirty-one CTD casts were made at locations on the seamount and along north–south and east–west transects extending up to 40 km from the summit. Data from the downcast of a representative CTD profile above the seamount are shown in *a* (whole water column) and *c* (upper 200 m detail). Zooplankton were obtained in 15 vertically stratified tows (nine samples per tow) at various locations on and near the seamount by a 1 m^2 MOCNESS (ref. 27) multiple opening and closing plankton net system using 335-μm mesh nets. Wet-weight zooplankton biomasses from a night-time tow series above the seamount are plotted in *b*. Relative fluorescence was measured by a Sea Tech *in situ* fluorometer and % light transmission by a Sea Tech 25-cm beam transmissometer, both attached to the MOCNESS. Representative profiles are shown in *b* and *e*. Fluorescence and light transmission data shown in *b* were smoothed with a five-point running mean. Fluorescence data in *d* were smoothed with a three-point running mean. Chlorophyll *a* and phaeophytin values (*d*), from water overlying the seamount, are means of two or three water samples per depth with two 500-ml aliquots per sample. Measurements were made fluorometrically[28] on water collected with Niskin bottles and filtered onto GFF filters. Abundances of diatoms, dinoflagellates and ciliate plus sarcodine protozoa (*e*) are means of three counts from water overlying the seamount (*n* per litre). Aliquots of 100 ml were drawn from Niskin bottles, preserved with Lugol's iodine, settled in settling chambers, and the entire sample was counted under an inverted microscope (magnification, ×170).

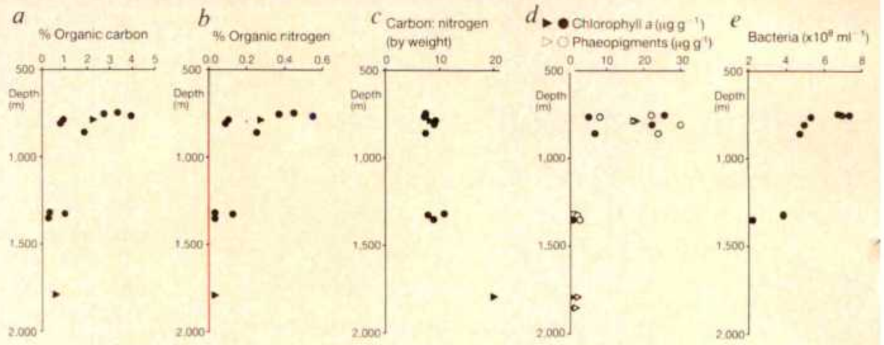

FIG. 2 Sediment organic matter properties. 1984 data, ▲; 1988 data, ●. Analyses of organic carbon (*a*), nitrogen (*b*), C/N (*c*), and chlorophyll *a* (▲, ●) and phaeopigments (△, ○) (*d*) were made on sediments collected by the submersible in tube cores and frozen at −70 °C. Organic C and N were analysed on the 0–2 cm vertical fraction. Sediments were homogenized, dried and ground, inorganic carbon was leached with 10% phosphoric acid for 24 h, then sediments were rinsed with organic-free distilled water, centrifuged, and the supernatant was decanted. The leaching process was repeated three times and remaining sediments were freeze-dried, weighed, ground and analysed on a Carlo Erba elemental analyser. Sediment chlorophyll *a* and phaeopigments were determined for 3 g sediment from the upper 1 cm using the spectrophotometric method[28] modified for sediments. Immediately on retrieval of tube cores, sediment bacteria (*e*) (1 ml from the top 1 cm) were sampled and preserved in 9 ml of 2.5% glutaraldehyde. Counts were made in the laboratory within two months of collection, using acridine-orange staining and epifluorescence microscopy[29]. There were significant differences (by analysis of variance) between upper (<770 m) and lower (770–900 m) summit locations for organic C ($P=0.015$) and N ($P=0.012$). Differences between summit (combined) and flank values were observed for organic C ($P=0.027$), organic N ($P=0.023$), chlorophyll *a* ($P=0.009$), phaeopigments ($P=0.001$) and bacteria ($P=0.004$).

Extraordinarily high densities (up to 19 individuals per m^2 in patches) occurred from 780–1,000 m, with maxima from 800–810 m (Figs 3*c*, *d* and 4*a*–*f*). Below 1,000 m, average megafaunal abundances (excluding xenophyophores) never exceeded 0.5 per m^2. This zonation did not correspond to the distribution of hard and soft substrates, and no hydrothermal activity was observed.

Abundance patterns of infauna, obtained in Ekman-style box cores, differed with size. Macrofauna (>300 μm) were rare above 750 m, with only low-oxygen-tolerant forms[16,17] present. Macrofauna increased greatly from the uppermost zone to 800–1,000 m (8,444 per m^2), then declined downslope (Fig. 4*g*). Meiofauna (smaller infauna), which are more tolerant than macrofauna to hypoxia[18], were most abundant at depths <750 m (202, 100 per m^2, $n=2$) (Fig. 4*h*). The nematode to harpacticoid copepod ratio, an indicator of oxygen stress[19], was 546:1 on the uppermost summit and declined to 1.3:1 on the flank (Fig. 4*i*).

Oxygen in near-bottom water samples obtained by submersible was 0.08–0.09 ml per litre ($n=2$,733–746 m) in the uppermost summit region of low megafaunal and macrofaunal abundance and 0.11–0.16 ml per litre ($n=3$,806–815 m) in the region of highest abundance. In five of six seamount CTD casts, oxygen was lower at 730 m than at 800 m, but there was considerable overlap in the concentration range measured at these two depths. Diurnal and semidiurnal temperature fluctuations (0.5 °C) occurred at the summit, indicating that internal tides were displacing water masses. This suggests that benthic organisms on the summit were exposed to periodically varying oxygen rather than to constant oxygen concentrations. Physiological exclusion of benthos at dynamic oxygen interfaces may therefore be a function of amplitude and temporal variation as well as absolute oxygen concentrations.

Very low plankton abundances are associated with oxygen minimum zones in the water column in parts of the Arabian Sea and the eastern Pacific, but only where oxygen is less than about 0.15 ml per litre throughout a broad depth interval[1,2], similar to the Volcano 7 region. At locations with more oxygen, plankton biomass may not be reduced, although distributions may be altered[1,20–22]. For midwater crustaceans, an oxygen concentration of 0.13 ml per litre is a critical lower oxygen limit for

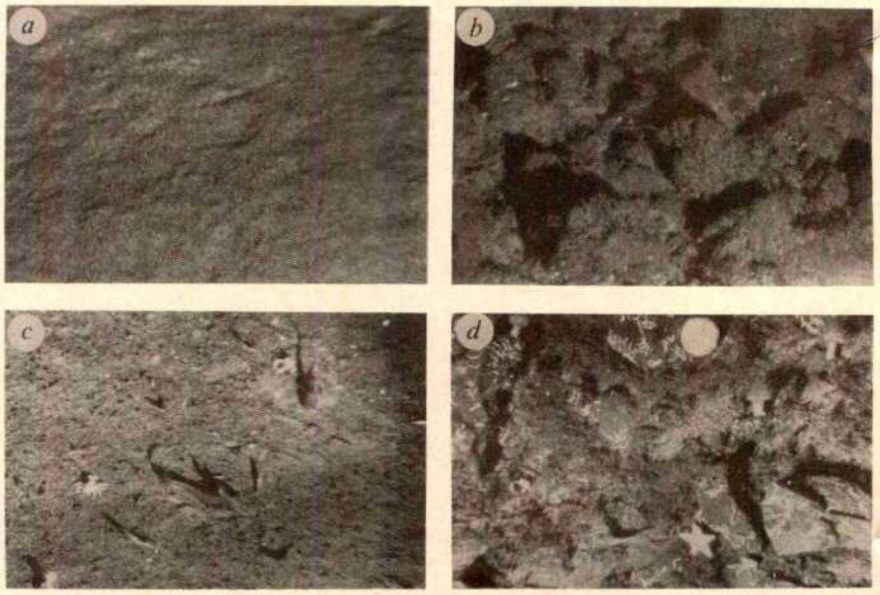

FIG. 3 Representative photographs of the upper summit (*a*, *b*; 744 and 734 m respectively) and lower summit (*c*, *d*; 770 and 780 m respectively). *a* and *c* show foraminiferal sand substrate, whereas *b* and *d* show rocky and mixed substrate. The only megafauna on the upper summit (*a*, *b*) were occasional rattail fish (*Nezumia liolepis*, shown in *c* and *d* but not in upper summit photographs) and solitary sessile coelenterates under rocks (White dots in *b*). Rock surfaces appear fuzzy (*b*, *d*) because of dense mats of an unidentified tube-building protozoan. Dark green detrital material was visible on rocks and sediments. Upper summit (*a*) meiofauna included large numbers of nematodes. The most abundant macrofauna were an aplacophoran (family Lepidomeniidae), a paraonid polychaete (*Cirrophorus lyra*), a dorvilleid polychaete (*Protodorvillea* sp.) and a few pogonophorans. A variety of abundant megafauna occurred on both rocks and sediments on the lower summit (*b*, *d*). Rattail fish (*N. liolepis*), galatheid crabs (*Munidopsis c.f. hystrix*), shrimp, starfish, sponges, anemones, ophiuroids and serpulid worm tubes (*Hyalopomatus marenzelleri*) are some of the organisms in *c* and *d*. Here, dark green or green-grey flocculent material occurred in depressions. Infaunal densities were high for sand substrates (>8,000 per m^2), and dominant infauna included cirratulid, sabellid and dorvilleid polychaetes and burrowing anemones. These photographs were taken with a hand-held 35-mm camera from inside the *Alvin* submersible and show an area of 2–3 m^2. For scale, the galatheid crabs are about 5 cm from claw tip to tail.

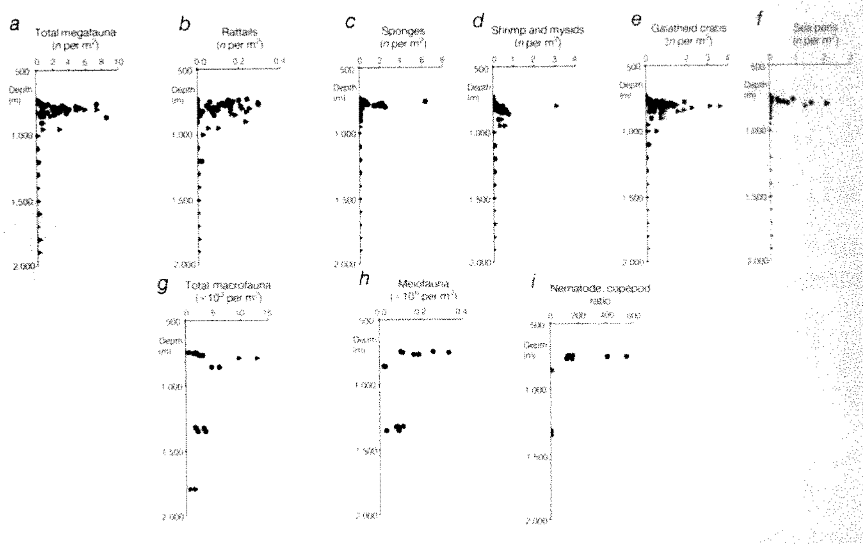

FIG. 4 Benthic faunal densities on the summit and flank as a function of water depth. 1984 data, ▲; 1988 data, ●. Megafaunal abundances were quantified from 1988 *Alvin* photographs (external hull-mounted camera) using a perspective grid to calculate surface area, and from 1984 *Angus* camera sled photographs, in which area was calculated as 1.25 sled altitude ×1.8 sled altitude. Mean faunal densities are shown for 10-m depth intervals 730–850 m, for 50-m intervals 850–1,000 m, and for 100-m intervals 1,000–2,000 m. Data from photographs taken on different parts of the seamount (at comparable depths) are plotted separately. Maximum densities of major taxa (1984 photographs) were 4.3 per m^2 for sponges, 9.7 per m^2 for galatheid crabs, 7.3 per m^2 for combined shrimp and mysids, 1.8 per m^2 for rattail fishes and 4.9 per m^2 for sea pens. For analysis of variance statistics, the upper summit includes data from 730–770 m and the lower summit, from 770–1,000 m. *a*, Total megafauna, excluding xenophyophores and the solitary coelenterates attached to rock bases on the upper summit. These groups were excluded because they were not reliably visible in *Angus* photographs. There were significant differences between the upper and lower summit ($P=0.036$) and the summit and flank ($P<0.001$). *b*, Rattail fishes (*N. liolepis*). Fish were ~15–20 cm long. There were significant differences between the upper and lower summit ($P=0.033$) and between the summit and flank ($P<0.001$). *c*, Total sponges. At least three unidentified species were present. There were significant differences between the summit and flank ($P=0.020$). *d*, Combined shrimp (*Heterocarpus nesisi* and *Benthescymus altus*) and mysids (*Boreomysis* sp.). Nearly significant differences were observed between summit and flank sites ($P=0.066$). *e*, Galatheid crabs (*M. c.f. hystrix*). Crabs are ~5-cm long. There were significant differences between upper and lower summit sites ($P=0.003$) and between summit and flank sites ($P<0.001$). *f*, Unidentified sea pens. These were present only on soft substrates and were ~15-cm long. *g*, Total macrofauna retained on a 300-μm screen. Samples were taken in Ekman-style box cores (196 cm^2 surface area) to a depth of 15 cm. Samples were preserved on board ship in 10% buffered formalin and later transferred to alcohol and sorted in the laboratory. Each data point represents macrofauna from one box core converted to *n* per m^2. There were significant differences between upper and lower summit sites ($P=0.003$) and between summit and flank sites ($P=0.051$). *h*, Total meiofauna (excluding foraminifera) retained on a 63-μm screen. Samples were taken from one subcore (49 cm^2 surface area) within each box core, processed as described above. Only the upper 5 cm of sediment were sorted for meiofauna. There were significant differences between the upper and lower summit ($P=0.044$). *i*, Nematode to harpacticoid copepod abundance ratios in the meiofauna cores. There were significant differences between summit and flank sites ($P=0.019$).

aerobic metabolism, although some vertical migrators can transit narrow low oxygen zones, or live anaerobically or at very low metabolic levels (in diapause, for example) for periods of time[23]. A pronounced oxygen-minimum zone that is vertically broad is therefore an effective barrier for most plankton. In this situation, the use of particulate material in midwater would be reduced, presumably enhancing benthic particulate fluxes.

Other interactions between the seamount and flow field could also mediate effects of the oxygen minimum. The high regional productivity of the eastern tropical Pacific[13] could be supplemented near Volcano 7 by topographically induced upwelling[24], which could contribute to the particle flux to the benthos. But, no definitive evidence of upwelling into the euphotic zone (which already extended below the mixed layer) was observed. Current acceleration over topographic features is an alternative process that may enhance local abundances of suspension-feeding megafauna on seamounts[25]. Although high abundances of some species occurred on ridges, the general megafaunal abundance peak on the summit of Volcano 7 did not correspond to exposed topography and included many non-suspension-feeding taxa.

We hypothesize that the striking benthic zonation on Volcano 7 is due to effects of the oxygen-minimum operating both in the water column (by enhancement of undegraded particulate food supply) and on the benthos (by exclusion of hypoxia-sensitive taxa). Oxygen minima also intersect the sea floor on continental slopes, as in the Arabian Sea[5], and the distinctive benthic faunal gradients produced by these interactions could be preserved in the palaeoceanographic record[4,6]. ☐

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Evolution of the MHC class I genes of a New World primate from ancestral homologues of human non-classical genes

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THE products of the classical human major histocompatibility complex (MHC) class I genes (*HLA-A, -B, -C*) are highly polymorphic molecules that bind peptides and present them to T lymphocytes[1–4]. The non-polymorphic, non-classical MHC class I gene products (*HLA-E, -F, -G*) are not restricting elements for the majority of T lymphocytes[5–7]. The evolutionary relationship of the non-classical and classical MHC class I genes is unclear. Here we present the cloning and sequencing of the MHC class I genes of a New World primate, the cotton-top tamarin (*Saguinus oedipus*). The expressed MHC class I genes of this species are more closely related to the human non-classical *HLA-G* (ref. 7) gene than they are to genes of the human classical *HLA-A, -B,* and *-C* loci. These observations imply that classical and non-classical genes do not necessarily constitute mutually exclusive groups over evolutionary time.

In contrast to other species[8], the MHC class I diversity of the cotton-top tamarin is limited[9]. Only 11 different MHC class I molecules were found in 79 unrelated tamarins (data not shown). To investigate the reasons for this limited MHC class I diversity, we have cloned full-length MHC class I complementary DNA from tamarins using conventional techniques[10] and the polymerase chain reaction[11] (PCR). Fortunately, all 11 tamarin MHC class I genes were expressed by the lymphocytes of three animals (numbers 100-75, 779-76 and 46-85). MHC class I cDNAs were isolated from these three animals and transfection of these cloned cDNAs into COS cells demonstrated that we had cloned all of the previously defined expressed tamarin genes (Fig. 1).

To ensure that we had isolated all of the expressed tamarin MHC class I genes, another set of primers was used to amplify exons 2 and 3 from RNA and DNA from animal 100-75. Only the three previously isolated cDNAs were amplified from RNA from this animal. In screening genomic clones, the three previously characterized cDNAs and three additional genes (*N1, N2, N3*) were defined (data not shown). *N2* was identical to cDNA 3 that had previously been cloned from animal 779-76. It is a gene with a premature termination codon in the cytoplasmic domain that was not expressed on the surface of lymphocytes from either animal 100-75 or 779-76. Both *N1* and *N3* contained deletions that would result in a premature termination codon or disrupt the folding of an MHC class I molecule (data not shown), indicating that they are pseudogenes. Thus, PCR amplification from genomic DNA did not reveal any additional intact tamarin MHC class I genes.

Analysis of the inter-locus variability in the human MHC class I genes shows that genes differ one from another by 50–100 bases in exons 2, 3 and 4. Intra-locus variability ranges from 1–50 bases in the same region[12]. Nucleotide sequences of the tamarin MHC class I genes were statistically less variable than those of human and mouse (Table 1). Accordingly, the predicted amino acid sequences of the tamarin MHC class I molecules were quite similar (Fig. 2). This similarity was especially striking in the transmembrane domain, in contrast to the highly variable MHC class I transmembrane domains of other species.

In phylogenetic trees[13] of exons 1-3, 4-7 and 1-7 (Fig. 3*a–c*), all but one of the tamarin cDNAs clustered with the human non-classical *HLA-G* MHC class I gene. The tamarin cDNA 3 was unique in that it was quite closely related to *HLA-F* (ref. 6). The presence of *HLA-G*- and *HLA-F*-specific substitutions in the predicted amino-acid sequences of the tamarin MHC

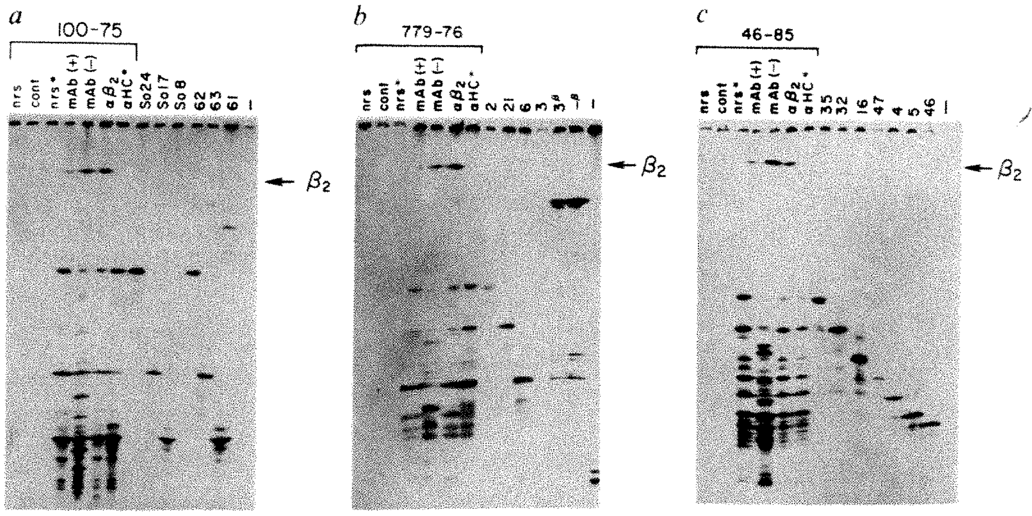

FIG. 1 Expression of seventeen cDNAs cloned from the lymphocytes of three cotton-top tamarins. *a*, PCR amplification of cDNA synthesized from RNA was used to clone full-length tamarin MHC class I cDNAs. Three MHC class I heavy chains and β_2-microglobulin (β_2) were precipitated[28] from the lysate of a biosynthetically labelled, EBV-transformed B-cell line from animal 100-75 using the monoclonal antibody BB7.7 (mAB). Precipitates were either left untreated (−) or sialic acid residues were removed with neuraminidase (+). A rabbit antiserum reactive with human β_2-microglobulin ($\alpha\beta_2$) and one reactive with denatured human heavy chains (αHC) also precipitated the same three tamarin MHC class I molecules. Normal rabbit serum (nrs) and a monoclonal antibody (cont) reactive with an irrelevant determinant were used as controls. Lysates were denatured (*) in some instances. Three cDNAs cloned from a cDNA library made from an EBV-transformed cell line from animal 100-75 (So24, So17 and So8)[10] and three cDNAs amplified by PCR[11,29] from cDNA synthesized from total cellular RNA isolated from the same cell line (62, 63 and 61) were transfected into COS cells. The sequences of the three PCR-amplified cDNAs (62, 63 and 61) differed by only 1 of 3,000 nucleotides from the sequences of those that were generated using standard cDNA cloning techniques (So24, So17 and So8). As a control, COS cells were also transfected with the mouse CD20 molecule (−). *b*, COS cells were transfected with four different cDNAs (2, 21, 6 and 3) isolated by PCR amplification of RNA from lectin-stimulated peripheral blood lymphocytes (PBL) from animal 779-76. MHC class I molecules from COS cells transfected with cDNA 3 were also precipitated with an antiserum reactive with human β_2-microglobulin (β). *c*, COS cells were transfected with seven different cDNAs (35, 32, 16, 47, 4, 5, and 46) isolated by PCR amplification of RNA from lectin-stimulated PBL from animal 46-85.

```
Leader peptide
                -10
Consensus   LLLLLSGALVLTETWA
So-8        ----------------
So-5        ----------------
So-32       ----------------
So-47        -----------D---
So-6        ----------------
So-16       ---------A------
So-17       ----------------
So-21       ----------------
So-4        -----P----------
So-46       ----------------
So-24       ---------SV-----
HLA-G       -F-------T------

So-3        -----L---T------
HLA-F       ---------A--D---

HLA-A,B,C   ---------A------
HLA-E       ------E--A--Q---

Alpha 1
            1       10        20        30        40        50        60        70        80        90
Consensus   GSHSMRYFSTSVSRPGRGEPRYIEVGYVDDTQFVRFDSDAASPRMEPRAPWMEQEGPEYWEEETRRAKAAAQTFRVNLQTLLGYYNQSEA
So-8        --------E--------------S-----------------------------------------KG--D------D-------------
So-5        --------E--------------S-----------------------------------------KG--D------G-------------
So-32       --------K--------------S---------------------------------D---K---T------------------------
So-47       --------E------------------------------------------------D---K----------------------------
So-6        --------K--------------S-----------------------------------------T----------D-------------
So-16       ----------A----------F-A-----------------R-----------------------KV--H--ID-MD-----------Q-
So-17       ----------A------E-------------------------------Q-V-K--R-------Q----F----------A---------
So-21       ----------T---------------------------N------------V------------Q----L------------R-----GV
So-4        ----------A------E---F-N--------------H--R----------------------LK--DW---Y----------------
So-46       ----------AM---------------------------------------V-K--R-------Q----F---D------A---------
So-24       ----------T----------------------------------------V--------VLQ--NS--S--------------------
HLA-G       ---------AA---------AF-AM--------------S-C---------V-------------NT--H---D-M-----R--------
                    △ △          RY                            △        ▲△  △△   △△    ▲△ ▲
So-3        ----L-----AM----------▾G-------L-------DP----------V---R-----DQ--VV--N---D--K-RK--R------G
HLA-F       ----L-----A------------A-E-------L-------I------E--V-----Q---WT-GY---N---D--A-RN--RR------
                ▲   △ △          ▲                             △        ▲        △△    △     △△
HLA-A,B,C   --------Y------------F-A---------------------------I--------DR--QIV--QS--D-ES-R--R--------
HLA-E       ----LK--H------------F-S-------------N-------V----------S---DR---S-RDT--I-----R--RR-------

Alpha 2
                   100       110       120       130       140       150       160       170       180
Consensus   GSHTIQWMYGCDLGPDGRLLRGYEQHAYDGKDYIALNEDLRSWTAADVAAQITQRKWEAANAAERTRAYLEGTCVEWLHRYLENGKETLQRA
So-8        -----------------------D---------------------T---------------E------------------------------
So-5        -----------------------H-Y-----------------------------------E------------------------------
So-32       --------S----------------------------------------------------S----------M-----L-------------
So-47       --------------------------------------------------------------------------------------------
So-6        --------S--------------H-Y---------------------A---------AE-----GM------V-----L-------------
So-16       ------V----------------D------------------------------------------------M-----L-------------
So-17       ------M-S--------------D----------S--------------------------E------------------------------
So-21       -----------Y-----------Y-----------------------L----------------EW--------------------------
So-4        ----L---S---------F------Y--------------------------------------GM--------------------------
So-46       -------------------------L--------------------------------------EW--------------------------
So-24       -F---------------------H-Y---------------------A---------AE-----GM------------R-H------M----
HLA-G       S---L---I-----S----------Y-------L-------------T----SK--C----V--QR---------------------M----
                  △     △          ▲                                    ▲                 ▲        ▲   ▲
So-3        ----L-GTN---V-----F----H----------S------------T-------MY-SEKY--EFST--K-G-------------------
HLA-F       ----L-G-N---M----------H----------S------------TV------FY--EEY--EF-T----E-L-L-R-------------
                  ▲ ▲                △        ▲                     ▲  ▲ ▲  ▲▲ ▲
HLA-A,B,C   ----L-R-----V----------H-Y---------------------T------------RV--QL------------R-------------
HLA-E       ----L---H--E----R-F------F-------LT----------V-T----SEQ-SND-SE--HQ-----D-------K---K-----LHL

Alpha 3
            183  190       200       210       220       230       240       250       260       270
Consensus   EPPKTHVTHHPVSDHEATLRCWALGFYPAEITLTWQRDGEDQTQDMELVETRPAGDGTFQKWAAVVVLSGEEHRYTCHVQHEGLPEPLTLRW
So-8        --------------------------------V---------------------------------------Q-------------------
So-5        --------------------------------V---------------------------------------Q-------------------
So-32       --------------------------------------------------------------------------------------------
So-47       ------------------------------------W-------------------------------------------------------
So-6        --------------------------------------------------------------------------------------------
So-16       ------M-------------------------------------------------------------------------------------
So-17       ------------------------------------------------------T-N-----------------------------------
So-21       ------------------------------------------------------T-N----------------I------------------
So-4        --------------------------------------------------------------------------------------------
So-46       -----------I--------------------------------------------------------------------------------
So-24       ------------------------------------------A-----------T-N----------------K-------------F----
HLA-G       D-----------F-Y----------------I-------------V---------------------P----Q---------------M---
                       △                                            △                            △
So-3        V---A--A---I-----I-------------M-------------T--------------------------Q-------------S-----
HLA-F       D---A--A---I----------------------------E----T---------------------P----Q------------Q--I---
                ▲  ▲                                                △
HLA-A,B,C   D----------I---------------------------------T----------R----------P----Q------------K------
HLA-E       -----------I------------------------Q---GH---T---------------------P----Q--------------V----

Transmembrane                                            Cytoplasmic Domains
            275  280       290       300       310             320         330       340
Consensus   EPPSQPTIPIMGIVAILAILGAVVTGAVVAAVMWRKKSS      DKKGGSYSQAA  RSDSAQGSDVSLTACK V
So-8        ---------------------V-----------------      -----------  ---------------- -
So-5        ---------------------V-----------------      -----------  ---------------- -
So-32       ---------------------------------------      -----------  ---------------- -
So-47       ---------------------------------------      -----------  ---------------- -
So-6        -----------------------------T---------      -----------  ---------------- -
So-16       ---------------------------------------      -----------  ---------------- -
So-17       -----------------------------T---------      -----------  ---------------- -
So-21       ---------------------------------------      -----------  ---------------- -
So-4        ---------------------------------------      -----------  ---------------- -
So-46       ---------------M-------------T---------      -----------  ---------------- -
So-24       ---------------------------------------      -----------  ---------------- -
HLA-G       KQS-L----------G-VV-A------A----L------      -
                 △    △    A     △  △        △ ▲         △
So-3        --S----VL----T▾GVVV---------    -----IWLGR   -RNR-------  M
HLA-F       -QSP------V----G-VV--------------------      -RNR-------  VT------G-----N- -
                 △           △  △△  △        △ ▲         △△▲▲
HLA-A,B,C   --S--S----V----G--V-AV--I--------C-R---      GG---------  S--------------- -
HLA-E       K-A-------V--I-G-VL--S--S-------I------      GG------K-E  W--------EHSL
```

FIG. 2 Predicted amino-acid sequences of the tamarin MHC class I cDNAs compared with the amino-acid sequences of *HLA-A*, *-B*, *-C*, *-E*, *-F* and *-G*. The sequence of the tamarin consensus is given and identity with this is indicated (–). The *HLA-A*, *-B*, *-C* consensus sequence is from 39 human sequences[1]. △ under the *HLA-G* sequence marks positions at which *HLA-G*-specific amino-acid substitutions are found in the tamarin but not the consensus *HLA-A*, *-B* and *-C* sequence. ▲ under the *HLA-G* sequence marks positions at which *HLA-G*-specific amino-acid substitutions are found in the tamarin, but not in any of 39 individual *HLA-A*, *-B -C* sequences[1]. △ under the *HLA-F* sequence marks positions at which *HLA-F*-specific amino-acid substitutions are found in So-3 but not in the *HLA-A*, *-B*, *-C* consensus sequence. ▲ under the *HLA-F* sequence marks positions at which *HLA-F*-specific amino-acid substitutions are found in So-3 but not in any of 39 individual *HLA-A*, *-B*, *-C* sequences[1].

METHODS. PCR from RNA was carried out as described previously[29]. The two sets of primers used in these studies have also been used to clone 21 cDNA *HLA-A*, *-B* and *-C* homologues from the lymphocytes of chimpanzee, pygmy chimpanzee, gorilla, orang-utan and gibbon and can therefore amplify *HLA-A*, *-B* or *-C* homologues (data not shown). Primer pairs used for amplification of full-length cDNAs from RNA were: 5′-GCAAGCTTATGACGGTCATGGCTCCCCGAACC-3′ and 5′-GCGAATTCAGTCCCACACAAGGCAGCTG-3′. Primer pairs used for amplification of exons 2 and 3 from RNA and genomic DNA were 5′-GCGAATTCGCTCCCACTCCATGAGGTATTTC-3′ and 5′-GCAAGCTTGCGCTGCAGCGTCTCCTTCCC-3′. Both strands of each PCR-generated isolate were sequenced[30] using oligonucleotide primers specific for MHC class I molecules and compressions resolved using dITP. These overlapping sequences were assembled (digitizer and software from IBI, New Haven, Connecticut) and aligned using programs from the University of Wisconsin Genetics Computer Group. To avoid PCR-generated errors[31] several copies of each isolated clone were sequenced as follows: So-24 (4), So-17 (2), So-8 (2), So-21 (3), So-6 (3), So-3/*N2* (4), So-32 (4), So-16 (3), So-47 (4), So-4 (7), So-5 (4), So-46 (3), *N1* (5) and *N3* (5). There are 8–11 MHC class I genes in the tamarin as indicated by Southern blot and hybridization of *Eco*RI- or *Hin*dIII-cut genomic DNA with MHC class I probes (data not shown).

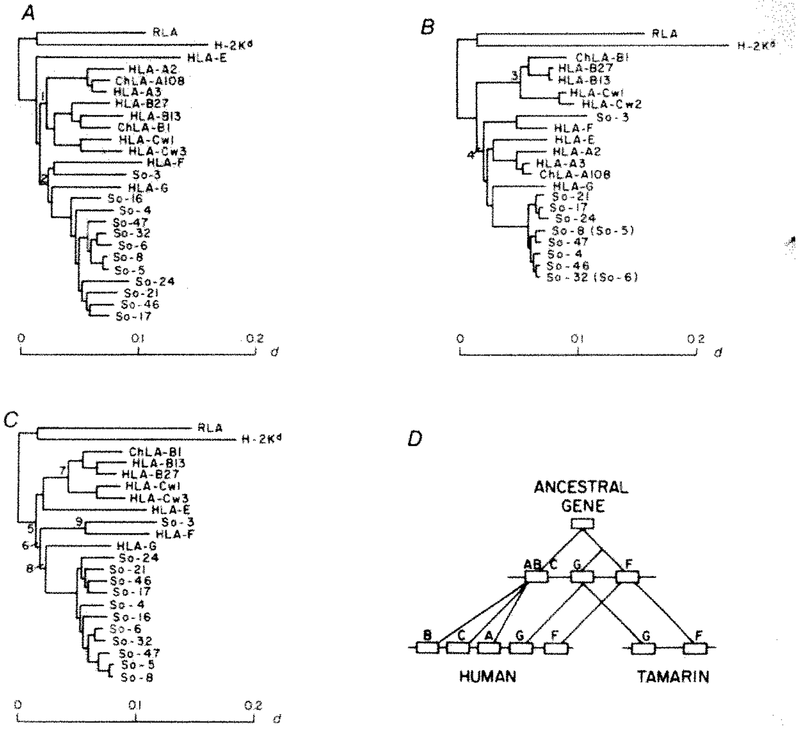

FIG. 3 The tamarin MHC class I genes are related to the *HLA-G* and *-F* non-classical genes. Trees were constructed for different regions of the MHC class I gene to clarify the relationship between the human and tamarin genes. This was necessary because the *HLA-A* locus, as it now exists, is the product of a recombination between an ancestral *HLA-A* locus and an *HLA-E*-like locus. As a result of this event (which antedated the human-chimpanzee divergence), the sequence of *HLA-A* (and of *ChLA-A*) 3' to the third intron is *HLA-E*-like and not closely related to other human classical MHC class I genes[32]. Phylogenetic trees based on nucleotide substitutions per site (*d*) among cotton-top tamarin and other primate MHC class I genes: *A*, tree for 591 aligned nucleotides from exons 1-3; *B*, tree for 414 aligned nucleotides from exons 4-7; *C*, tree for 1,005 aligned nucleotides from exons 1-7. In all cases rabbit (*RLA*) and mouse (*H-2K^d*) MHC class I genes are used as outgroups to establish the root of the tree. Significance of selected internal branch lengths was tested by the method of Li[33]. In *A*, the internal branch d_{12} (between the human classical gene cluster and the human non-classical/tamarin cluster) is significant at the 5% level (t = 2.05). In *B*, the internal branch length d_{34} (between the cluster containing tamarin genes, human non-classical genes and this region of the *A* alleles and the cluster containing *B* and *C* alleles) is significant at the 0.1% level (t = 4.10). In *C*, the internal branch d_{67} (between the cluster containing the tamarin genes, *G* and *F* and the cluster of human and chimpanzee classical genes) is significant at the 0.1% level (t = 4.61); the internal branch d_{89} (between the *F*/*So*-3 cluster and the cluster containing *G*, and the other tamarin sequences) is significant at the 0.1% level (t = 6.20); the internal branch d_{58} (between the *G*-human classical branch point and the *G*-tamarin branch point) is significant at the 5% level (t = 2.09); and the internal branch length d_{59} (between the *F*-human classical branch point and the *F*-So-3 branch point) is significant at the 0.1% level (t = 6.33). The fact that d_{58} and d_{59} are significant is evidence that *G*, *F* and the ancestor of human classical class I genes existed before the separation of Old and New World primates. *D*, Schematic representation of a hypothetical evolutionary history of tamarin and human MHC class I genes. This figure is not intended to represent relative chromosome positions of the various genes.

class I molecules supports the results of this phylogenetic analysis (Fig. 2). Gene trees based on the partial nucleotide sequences available for *N1* and *N3* were also constructed. These two genes were also related to the *HLA-G* locus (data not shown). Thus, all tamarin MHC class I genes have evolved from ancestral homologues of human non-classical MHC class I genes.

We could find no evidence for the presence of tamarin MHC class I gene products related to human HLA-A, -B or -C molecules. Antibodies against human MHC class I molecules precipitated only the three HLA-G-related products from a B-cell line from animal 100-75. Synthesis of a cDNA library from this cell line using conventional techniques[10] and PCR amplification using two different primer pairs also demonstrated that only these three MHC class I genes were expressed. As tamarins can mount CD8$^+$ cytotoxic T-cell responses[14], it is likely that their HLA-G-related MHC class I molecules are functional. These observations suggest that HLA-G-related MHC class I genes constitute the classical MHC class I genes of the tamarin. This implies that classical and non-classical MHC class I genes need not constitute mutually exclusive groups over evolutionary time.

TABLE 1 The variability of the expressed tamarin MHC class I genes is significantly lower than the variability of the human and mouse MHC class I genes

Species	Comparison no.	$d \pm$ s.e.m.	$d_s \pm$ s.e.m.	$d_N \pm$ s.e.m.
Cotton-top tamarin	55	4.7 ± 0.4	4.8 ± 0.8	4.7 ± 0.5
Human	66	8.7 ± 0.6*	13.9 ± 1.7*	7.1 ± 0.6*
Mouse	36	8.2 ± 0.5*	7.1 ± 1.0	8.6 ± 0.6*

Comparisons of mean numbers of nucleotide substitutions per site (d), per synonymous site (d_s) and per non-synonymous site (d_N); expressed as percentages in alpha-1, alpha-2 and alpha-3 domains (822 nucleotides) of cotton-top tamarin, human and mouse MHC class I molecules. d is estimated by the Jukes-Cantor method[24], d_s and d_N are estimated by the Nei and Gojobori method[25], standard errors are estimated by the Nei and Jin method[26]. See ref. 27 for human and mouse sequence references.

* Means for mouse and human that differ significantly (0.1% level) from the corresponding means for tamarin.

A phylogenetic analysis of human and tamarin MHC class I sequence data (Fig. 3) suggests that the last common ancestor of humans and tamarins had at least the following MHC class I genes: *G*, *F* and the ancestors of *A*, *B* and *C*. Both the tamarin MHC class I *G*-related and *F*-related genes appear to have diverged from the ancestral *G* and *F* genes after they had split from the ancestor of the *HLA-A*, *-B* and *-C* genes. This hypothesis is supported by the significant branch length (d_{58}, Fig. 3*c*) between *G* and the branch point of the ancestor of *HLA-A*, *-B* and *-C* (5) and *G* and the branch point of the tamarin *G*-related genes (8). The *F* gene also diverged from *G* after *G* had split from the ancestor of *A*, *B* and *C* as indicated by a similar significant internal branch length (d_{59}, Fig. 3*c*). Therefore, as both *G* and *F* diverged from *ABC* before the derivation of the tamarin genes and as *G* and *F* are both present in tamarins and humans, the MHC chromosome of the last common ancestor of humans and tamarins probably had genes for *G*, *F* and *ABC* (Fig. 3*d*).

Hybridization studies[15] and sequence analyses[16] had suggested that non-classical MHC class I genes may not be conserved in different orders of mammals. The isolation of a chimpanzee *HLA-F* cDNA[12], however, demonstrates that non-classical loci can be shared by closely related mammalian species. The finding in the tamarin of a gene related to *HLA-F* that is not expressed shows that non-classical MHC class I loci can be preserved for much longer than was previously thought.

The remarkable lack of variation and polymorphism at the MHC class I loci of the tamarin may explain, in part, the extreme susceptibility of these New World primates to a variety of viral pathogens. Tamarins succumb to a fatal lymphoproliferative syndrome when infected with Epstein-Barr virus (EBV), *Herpesvirus saimiri* and *H. ateles*[17,18]. They also die following infection with Rous sarcoma virus, measles and parainfluenza viruses[17].

As individual MHA class I glycoproteins can probably bind and present only a restricted number of viral peptides to T cells, reduced variability in the species' MHC class I glycoproteins may render tamarin MHC class I molecules unable to bind a diversity of viral peptides. This may leave the tamarin susceptible to fatal infections by pathogens to which it is not exposed in its natural environment.

Finally, it is interesting to speculate as to the evolutionary pressures that might have selected for deletion of the *HLA-A*, *-B*, and *-C* in the tamarin and utilization of *HLA-G*-related MHC class I genes. Most tamarins are born as stable bone marrow-chimaeric fraternal twins[19]. MHC class I similarity between genetically disparate, coexisting cell populations would facilitate this chimaerism. Indeed, it has recently been demonstrated that cells at the interface of the human maternal and fetal circulations in the extravillous trophoblast do not express detectable classical HLA class I molecules[20,21]. Rather, these fetal cells express products of the non-polymorphic, non-classical *HLA-G* genes[22,23]. Utilization of relatively non-polymorphic MHC class I genes may be a mechanism by which MHC class I functions can be served with a minimum stimulation of alloreactivity. □

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Inhibition by brefeldin A of presentation of exogenous protein antigens to MHC class II-restricted T cells

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PEPTIDES bound to class I or class II major histocompatibility complex (MHC)-encoded molecules are ligands for the antigen-specific T-cell receptor of T-cells carrying the CD8 and CD4 antigens, respectively[1]. MHC class I-restricted T cells generally recognize peptides derived from processing of endogenously synthesized cellular antigens, whereas class II-restricted T cells usually recognize peptides derived from exogenous antigens entering antigen presenting cells. Accordingly, two separate pathways of antigen processing and presentation have been proposed[2,3]. The fungal metabolite brefeldin A (BFA)[4], an inhibitor of protein transport from the endoplasmic reticulum[5–7], inhibits presentation of endogenous antigens for MHC-restricted T-cell recognition[8]. The selectivity of BFA activity has been inferred to reflect presentation of a given antigen processed through the cytosolic or the endocytic route[9,10]. Here we show that BFA also greatly inhibits the presentation of exogenous protein antigens by MHC class II molecules to T cells, indicating a broader effect of this drug on antigen presentation and an additional similarity between the two processing pathways. As cycloheximide, a protein synthesis inhibitor, also inhibits presentation of protein antigens to class II-restricted T cells, the data indicate that peptides generated by processing of exogenous proteins bind to newly synthesized class II molecules for presentation to T cells.

To test the effect of BFA on the presentation of exogenous protein antigens to MHC class II-restricted T cells, LK-35.2 cells (a B-cell hybridoma expressing I-$A^{k,d}$ and I-$E^{k,d}$ molecules) were treated with BFA during a 4-h pulse with hen egg-white lysozyme (HEL) or chicken ovalbumin (Ova). After washing these antigen presenting cells (APC), antigen presentation to the HEL-specific T-cell hybridoma 1H11.3 or Ova-specific hybridoma DO11.10 was assessed by measuring T-cell growth factor (interleukins 2 and 4) production. Incubation of APC with 3 μM BFA abrogates their capacity to present exogenous protein antigens (Fig. 1*a*, *b*). If, under the same conditions, APC are pulsed with the HEL peptide 105–120 (recognized by hybridoma 1H11.3 with I-E^{d} molecules[11]) or with the Ova peptide 323–339 (recognized by hybridoma DO11.10 with I-A^{d} molecules[12]), BFA has no effect. Titration of interleukins produced by peptide-activated T-cell hybridomas also did not show any effect of BFA on antigen presentation (data not shown). These results demonstrate that BFA inhibits the presentation of exogenous soluble proteins recognized by T cells with MHC class II molecules, but has no effect on the ability of APC to present synthetic peptides.

The effect of BFA on antigen presentation can be partially overcome by increasing the concentration of protein antigen during pulsing, using T-cell hybridomas responding to low antigen concentration (Fig. 1*c*–*h*). A similar effect is also exerted on LK-35.2 cells by cycloheximide, although this was not entirely predicted by previous work[13,14]. In fact, a remarkable concordance between BFA and cycloheximide in the inhibition of exogenous antigen presentation is observed. These results suggest that a less efficient, secondary pathway for class II-restricted antigen presentation, independent of *de novo* protein synthesis or of protein egress from the endoplasmic reticulum (ER), may be activated by pulsing the APC with high (at least 20-fold molar excess) protein antigen concentrations. Alternatively, the data may reflect incomplete inhibition of protein egress from the ER. Conversely, the lysosomotropic agent chloroquine inhibits antigen presentation at any concentration of protein antigen tested, indicating that BFA does not act as a lysosomotropic agent.

The inhibitory effect of BFA on the presentation of soluble proteins to class II-restricted T cells can be demonstrated in different types of APC, including B-hybridoma and B-lymphoma

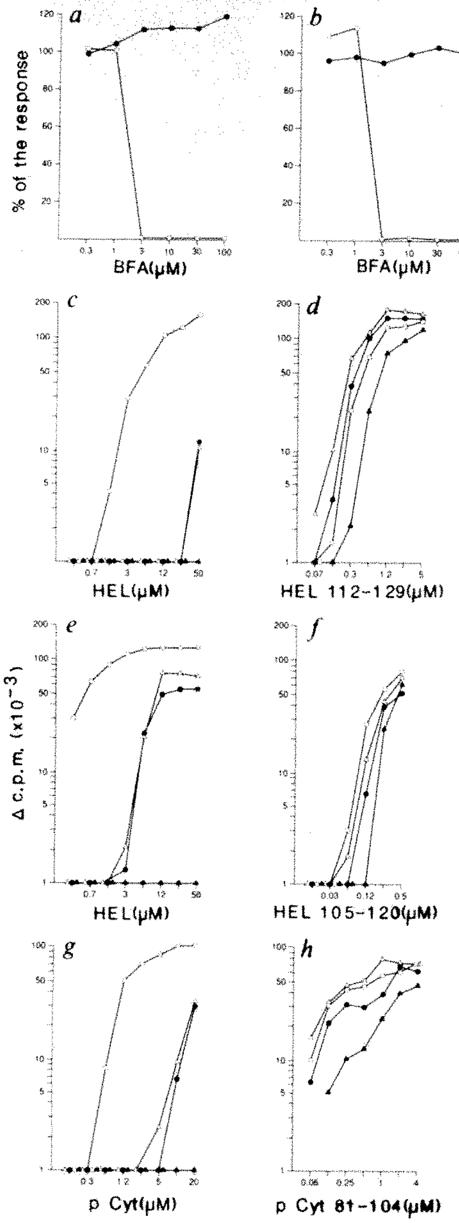

FIG. 1 Inhibition of antigen presentation by BFA. LK-35.2 APC (2.5×10^4 per well) were incubated with the indicated concentrations of BFA (Sandoz; M_r 280) and 15 min later antigen was added: *a*, 2 μM HEL (○) or 2 μM HEL peptide 105–120 (●); *b*, 250 μM Ova (○) or 1 μM Ova peptide 323–339 (●). After 4-h incubation at 37 °C, the APC were washed three times and 5×10^4 cells per well of hybridoma 1H11.3 (*a*) or DO11.10 (*b*) were added. After 24 h, antigen-specific T-cell growth factor production was determined by ^{3}H-labelled thymidine incorporation into CTLL cells (ref. 21). Data are presented as percentage of the c.p.m. (arithmetic mean of triplicate cultures) obtained in response to the protein antigen or to the peptide. Control responses in the absence and presence of protein and peptide were respectively: 660/75,578 and 515/81,181 (*a*); 528/54,904 and 568/93,175 (*b*). *c-h* LK-35.2 cells were incubated with 20 μM BFA (△), 350 μM cycloheximide (●) (Sigma), 200 μM chloroquine (▲) (Sigma), or left untreated (○). These compounds were added to APC 15 min, 2 h or 30 min, respectively, before addition of the indicated concentrations of protein antigens or synthetic peptides. After 4-h incubation with antigen, the APC were washed, fixed with 0.05% glutaraldehyde[21], washed again and the HEL-specific T-cell hybridomas 2C8.4, recognizing the HEL peptide 112–129 in association with I-A^k molecules[21] (*c*, *d*), 1H11.3 (*e*, *f*), or the pigeon cytochrome *c* (p Cyt)-specific T-cell hybridoma 2B4.11, recognizing the p Cyt peptide 81–104 in association with I-E^k molecules[22] (*g*, *h*), were added. The experiment was continued as in *a*, results are expressed as c.p.m. of ^{3}H-labelled thymidine incorporation into CTLL cells. Background proliferation of CTLL cells was 201 (*c*, *d*), 136 (*e*, *f*) and 150 (*g*, *h*) c.p.m.

METHODS. The synthesis, purification and analysis of HEL peptides and the establishment of T-cell hybridomas have been described previously[11,21]. Cultures containing 5×10^4 T-hybridoma cells and 2.5×10^4 LK-35.2 cells (American Type Culture Collection) were set up with or without antigen in 0.2 ml RPMI 1640 medium (Gibco) supplemented with 2 mM L-glutamine, 50 μM 2-mercaptoethanol, 50 μg ml^{-1} gentamicin and 10% FCS (Seromed). After 24 h of culture, 50 μl aliquots of supernatant were assayed for the presence of T-cell growth factors by ^{3}H-labelled thymidine incorporation in 10^4 CTLL cells[11].

cells (Fig. 2*a*), resident peritoneal adherent cells (Fig. 2*b*) and MHC class II-transfected L-cell fibroblasts (Fig. 2*c*). In the latter case, fibroblasts transfected only with I-E^d genes[15] or supertransfected with the invariant chain gene[16], were equally sensitive to BFA, suggesting that expression of the invariant chain does not influence the inhibition by BFA of protein antigen presentation to class II-restricted T-cells.

Addition of BFA to APC 1 h after HEL induces a much reduced effect on antigen presentation, and no effect 2 h after HEL, indicating that during this period a sufficient amount of protein had been processed to yield antigenic complexes stimulating an optimal T-cell response (Fig. 3*a*). Addition of BFA to APC any time after the HEL peptide 105–120, is ineffective (Fig. 3*b*). The inhibition of class II-restricted antigen presentation by BFA is rapidly and completely reversible, and washing the APC after 4-h incubation with BFA, just before the addition of HEL

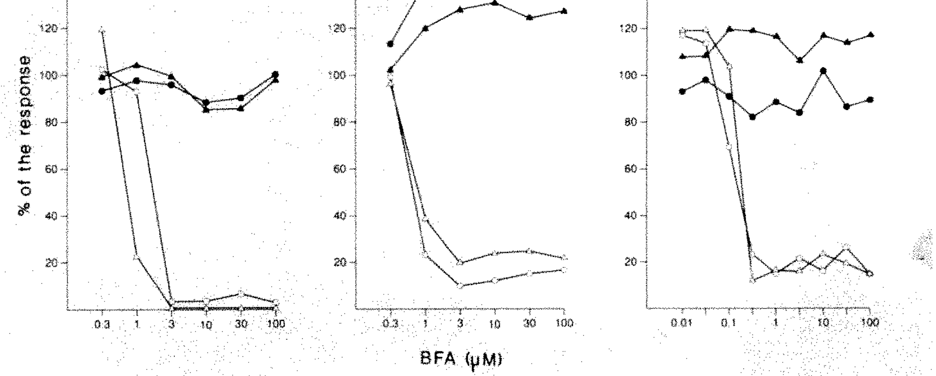

FIG. 2 Inhibition by BFA of the presentation of HEL by different APC. APC were incubated with the indicated concentrations of BFA and 15 min later 2 μM HEL (open symbols) or 2 μM HEL peptide 105–120 (closed symbols) were added. *a*, LK-35.2 (circles), A20 cells (triangles). *b*, Resident peritoneal cells enriched in macrophages by plastic adherence from BALB/c (circles) or DBA/2 mice (triangles). *c*, CA36.2.1 fibroblasts transfected with I-E^d genes[15] (circles) or CA36.2.1.li supertransfected with the invariant chain gene[16] (triangles). The experiment was then continued as in Fig. 1. Control responses of the I-E^d-restricted T-cell hybridoma 1H11.3 in the absence and presence of HEL and the HEL peptide 105–120 were respectively: 1019/134,776 and 861/142,914 (LK-35.2); 816/25,207 and 855/78,439 (A20); 711/45,344 and 435/31,413 (BALB/c); 863/61,174 and 671/46,062 (DBA/2); 285/36,898 and 346/95,105 (CA36.1.2); 360/21,515 and 256/76,398 (CA36.1.2.li).

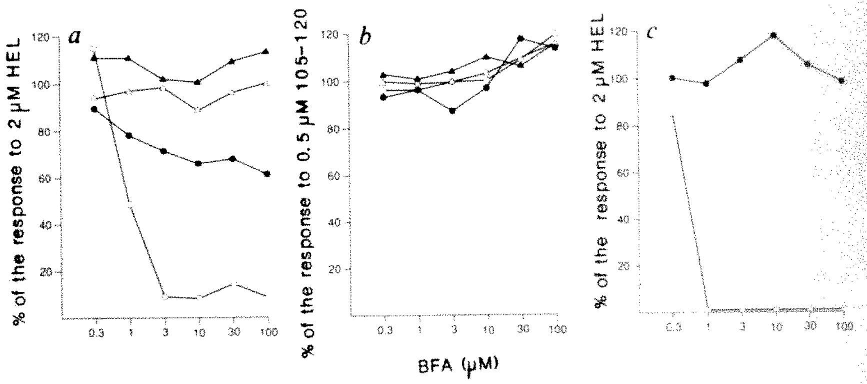

FIG. 3 The inhibition by BFA of HEL presentation is reversible and requires the presence of the drug during the antigen processing phase. *a,b*, LK-35.2 cells were incubated with 2 μM HEL (*a*), or 0.5 μM HEL peptide 105–120 (*b*) and the indicated concentrations of BFA were added 15 min before antigen (○), or 1 h (●), 2 h (△), 3 h (▲) after antigen. The experiment was then continued as in Fig. 1. Control responses of hybridoma 1H11.3 in the presence or absence of HEL and HEL peptide 105–120 were respectively: 745/164,340 and 816/165,197 c.p.m. *c*, LK-35.2 cells were incubated with the indicated concentrations of BFA and 15 min later 2 μM HEL was added. After 4 h, APC were washed, the I–E^d-restricted HEL-specific T-cell hybridoma 1E5.11 (ref. 11) was added, and the experiment continued as in Fig. 1 (○). Alternatively, LK-35.2 cells were incubated with BFA for 4 h, washed, pulsed with HEL for 4 h and the experiment continued as above (●). Control responses in the absence or presence of HEL were respectively 883 and 27,391 c.p.m.

and T-hybridoma cells, restores interleukin-2 (IL-2) production to control levels (Fig. 3*c*).

The effect of BFA on antigen presentation does not seem to be related to the amount of surface class II molecules expressed. No difference could be detected by surface immunofluorescence with anti-I-E^d antibody between untreated and BFA-treated cells (Fig. 4*c*), or in their antigen-presenting function, as peptide presentation to class II-restricted T cells is not affected by BFA treatment of APC.

But BFA affects the biosynthetic maturation of MHC class II molecules, as demonstrated by pulse-chase labelled LK-35.2 cells. Cells were immunoprecipitated with anti-I-A and anti-I-E monoclonal antibodies immediately after labelling and after a 3.5-h chase period. BFA retarded the biosynthetic maturation of both I-A (Fig. 4*a*) and I-E (data not shown) class II molecules, as lower relative molecular mass (M_r) α- and β-chain species were observed in BFA-treated (Fig. 4*a*; lanes 3, 4) but not in untreated cells (Fig. 4*a*; lanes 1, 2). These lower M_r species were sensitive to endoglycosidase H (endo H) treatment before and at the end of the 3.5-h chase period (Fig. 4*b*; lanes 6, 8), whereas class II molecules from untreated cells were sensitive to endo H treatment before the chase period, but acquired endo H resistance during 3.5-h post synthesis (Fig. 4*b*; lanes 2 and 4, respectively). The lower M_r I-A^k species present in BFA-treated cells before the chase period (Fig. 4*a*, lane 3 and *b*, lane 5) was converted to slightly larger species during the 3.5-h chase period (Fig. 4*a*, lane 4 and *b*, lane 7). Furthermore, an increased ability of the monoclonal antibody to immunoprecipitate I-A^k molecules after the chase period in BFA-treated cells is apparent (compare Fig. 4*a*, lanes 3, 4, and *b*, lanes 5, 7). This may represent continued processing of some of the carbohydrate chains of the I-A^k molecule during the BFA treatment. A similar situation has been described for the T-cell receptor α- and γ-chains, and was accounted for by the activity of *cis*-medial Golgi glycosidases and glycosyltransferases redistributed by BFA in the ER[6]. In contrast to the T-cell receptor α-chain, I-A^k molecules retain some sensitivity to endo H (Fig. 4*b*, lane 8). Thus, BFA treatment prevents complete maturation

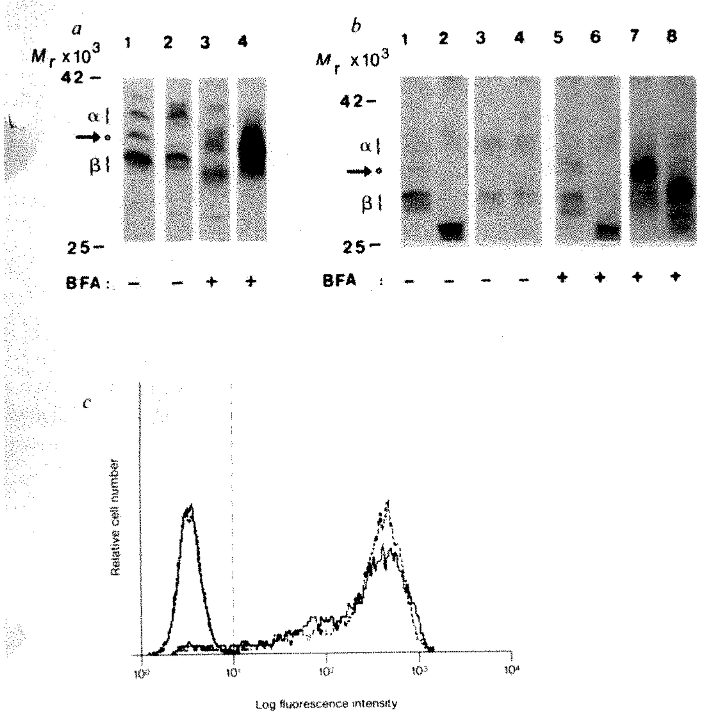

FIG. 4 Effect of BFA on the expression of class II MHC molecules. *a*, Anti-I–A^k immunoprecipitates from BFA-untreated (lanes 1, 2) or -treated (lanes 3, 4) cell lysates at 0 h (lanes 1, 3) or 3.5 h (lanes 2, 4) after start of chase period. *b*, Endo H sensitivity of class II molecules. Anti-I–A^k immunoprecipitates of lysates from 0 h (lanes 1, 2, 5, 6) and 3.5 h (lanes 3, 4, 7, 8) after the start of the chase period were digested with endo H. Lanes 1–4 and 5–8 represent immunoprecipitates from control and BFA-treated cell lysates, respectively. Mock- and endo H-digested immunoprecipitates are lanes 1, 3, 5, 7 and 2, 4, 6, 8, respectively. The upper and lower brackets to the left correspond to the position of the α- and β-chains of class II molecules, respectively, and the arrow to the position of the class II-associated invariant chain. The position of M_r standards are indicated. *c*, Cell surface expression of class II molecules. LK-35.2 cells were left untreated (solid line) or incubated with 100 μM BFA (broken line) for 4 h before staining with anti-I–E monoclonal antibody.

METHODS. *a,b*, LK-35.2 cells (2.5×10^6 ml^{-1}) were incubated with 10 μM BFA or left untreated and 15 min later metabolically labelled with 250 μCi ml^{-1} [^{35}S]methionine (Dupont, 1,100 Ci mmol^{-1}) in methionine-free medium containing 2.5% dialysed FCS for 30 min. Cells were then immediately harvested or chased for 3.5 h in complete medium, lysates were prepared from 5×10^6 cells per time point and immunoprecipitated as described[23] with the anti-I–A^k monoclonal antibody 10.2.16 or with an irrelevant antibody (not shown) and the resulting immune complexes washed as described[24]. Immunoprecipitates were analysed on 12% (*a*) or 10% (*b*) SDS-PAGE[25]. Immunoprecipitates were dissociated by heating for 5 min at 90 °C in 40 μl of 0.5% SDS. Half of the solubilized preparation was digested at 30 °C with 8 mU endo H (Genzyme) in 50 mM sodium phosphate buffer, pH 6.5, 0.05% SDS (lanes 2, 4) and the other half mock-digested (lanes 1,3). After 18 h, 10 μg RNA carrier transfer was added, the digest precipitated by adding four volumes of cold acetone, the precipitate centrifuged at 10,000*g* (10 min), and the resulting pellet analysed by SDS-PAGE. *c*, Cells were washed, incubated with biotinilated monoclonal antibody 14-4-4 (anti-I–E^d), washed, incubated with fluorescein isothiocyanate (FITC)-streptavidin and analysed using FACStar (Becton-Dickinson). The left peaks represent the fluorescence of cells not incubated with antibody.

of class II molecules in LK-35.2 cells, consistent with the BFA-induced inhibition of glycoprotein carbohydrate maturation due to failure of nascent proteins to leave the ER[5,6].

Our data demonstrate that the presentation of soluble exogenous proteins by class II molecules is, in contrast to earlier work[10], BFA-sensitive. The reason for this difference is not clear but could reflect the type of antigen, particulate or soluble, used. It has been suggested that BFA may not inhibit pigeon cytochrome *c* presentation by B-cell hybridomas to class II-restricted T-cells[8]. But the presentation of pigeon cytochrome *c* by LK-35.2 cells to the T-cell hybridoma 2B4.11 is clearly inhibited by BFA (Fig. 1*g*, *h*). It has also been suggested that BFA inhibits antigen presentation by macrophages but not by B-cell lymphomas[17]. In contrast, our data demonstrate a similar sensitivity to BFA for all the APC tested.

Different explanations could be envisaged for the BFA inhibition of exogenous antigen presentation to MHC class II-restricted T-cells: prevention of peptide generation, of peptide-class II association, or of egress of newly synthesized class II molecules from the ER. The first possibility is unlikely as BFA is probably not a lysosomotropic agent[5,8], and does not inhibit aspartyl, serine or metalloproteases (H. Wagner, unpublished data). But BFA might inhibit processing of exogenous antigens if processing requires the transport of newly synthesized proteolytic enzymes into the endosomes. The second possibility is not true for synthetic peptides, which can also directly bind to surface class II molecules. Although BFA may inhibit the association of intracellular peptides to class II molecules, peptides can still bind to MHC class I molecules in BFA-treated cells[18]. As BFA blocks protein egress from the ER, the third possibility would predict that encounter, likely in a *trans*- or post-Golgi location, of newly synthesized class II molecules with peptides derived by processing of exogenous proteins, could be prevented by BFA treatment of APC. Cycloheximide also inhibits antigen presentation, indicating that a continuous flow of newly synthesized MHC class II molecules from the ER is required for efficient presentation to T-cells of peptides generated by processing of exogenous proteins. This requirement does not contradict the hypothesis that MHC class II/peptide complexes, after being expressed on the cell surface, may recycle to endocellular organelles allowing peptide exchange to take place[19,20]. □

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Tyrosine phosphatase CD45 is essential for coupling T-cell antigen receptor to the phosphatidyl inositol pathway

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STIMULATION of T lymphocytes through their antigen receptor (T-cell receptor; TCR) results in the activation of a tyrosine kinase[1,2] and the generation of phosphatidyl inositol (PtdIns)-derived second messengers[3–5]. Several reports have indicated that CD45, a haematopoetic cell-specific surface glycoprotein with tyrosine phosphatase activity in its cytoplasmic domain[6–10], is important in lymphocyte activation[11–14]. To examine the possibility that CD45 might influence proximal signal transduction events through the TCR, we have isolated a variant of the human T-cell leukaemic line, HPB-ALL, which fails to express this phosphatase. Unlike cells expressing CD45, stimulation of the TCR in the CD45-negative cell does not result in PtdIns-derived second messengers. Reconstitution of CD45 expression restored early signalling events through the TCR. To localize the site of CD45 action, the human muscarinic type 1 receptor, which also activates the PtdIns second messenger pathway[15,16], was transfected into the CD45-negative cell. Although stimulation of the TCR failed to generate PtdIns-derived second messengers, there was normal activity of the PtdIns pathway when human muscarinic receptor type 1 was stimulated, despite the absence of CD45. These data indicate that CD45 influences a cellular component that is essential for effective coupling of the TCR to the PtdIns second messenger pathway.

We screened a number of human T-cell leukaemic lines for surface expression of CD45. One of the lines screened, HPB-ALL, stained heterogeneously with a panel of monoclonal antibodies (mAb) directed against CD45. Subcloning resulted in the isolation of several CD45-negative and CD45-positive HPB-ALL variants. Figure 1*a–d* shows flow cytometry histograms of representative isolates of these cells stained for surface expression of the TCR complex and CD45. In contrast to a previously described mutant cell line deficient in CD45 surface expression[11], northern blot analysis revealed no messenger RNA for CD45 in our negative variant, HPB.45.0 (data not shown). HPB.45.0 expresses significantly more TCR than does the CD45-positive variant, HPB.45.1 as assessed with anti-CD3 mAb.

We next examined the possible role of the CD45 tyrosine phosphatase in early events associated with TCR-mediated signal transduction. The ability of anti-CD3 mAb to activate the PtdIns signal transduction pathway was evaluated by measuring changes in cytoplasmic free Ca^{2+} ($[Ca^{2+}]_i$) (Fig. 2). In HPB.45.1, the cell expressing CD45, a rapid and large increase in $[Ca^{2+}]_i$ was seen in response to the activating anti-CD3 mAb, Leu 4 (Fig. 2*b*), similar to increases in $[Ca^{2+}]_i$ previously reported for HPB-ALL and other T cells[3,17,18]. By contrast, no detectable increase in $[Ca^{2+}]_i$ was observed in the CD45-negative cell HPB.45.0 (Fig. 2*a*), even though this cell expresses over an order of magnitude more TCR than the CD45-positive cell. That the cells were adequately loaded with the calcium-sensitive fluor, Indo-1, was shown by the response to the calcium ionophore, ionomycin.

The increase in $[Ca^{2+}]_i$ seen after stimulation of the TCR is due to the generation of soluble inositol phosphates by the hydrolysis of phosphatidyl inositol 4,5-bisphosphate ($PtdIns(4,5)P_2$) (refs 4, 19). We therefore studied the ability of anti-CD3 mAb to induce $PtdIns(4,5)P_2$ breakdown in the CD45-negative and -positive cells. Figure 3 depicts the result of such an experiment where inositol phosphates were measured at various times after anti-CD3 stimulation of HBP.45.0 and HPB.45.1. As expected from the observed increases in $[Ca^{2+}]_i$, the CD45-positive cells consistently generated an increase in soluble inositol phosphates, whereas the CD45-negative cells showed no such response.

Because activating stimuli alter expression of CD45 in T cells[20], we tested the effect of phorbol myristyl-13-acetate (PMA) treatment on CD45 levels in the CD45-negative cells. Following 24 h of PMA incubation, there was low but detectable induction of expression of CD45, peaking at ~48 h (Fig. 1*e*, *f*), and correlating with induction of CD45 mRNA (data not shown). To our knowledge, this is the first example of induction of the CD45 gene due to an external stimulus. The PMA-treated cells were stimulated with anti-CD3 mAb and analysed for increases in $[Ca^{2+}]_i$. Figure 2*c* shows a rapid and sustained increase in $[Ca^{2+}]_i$ seen in response to the Leu 4 mAb in PMA-treated HPB.45.0 cells. As expected, analysis of soluble inositol phosphates after anti-CD3 stimulation revealed substantial increases in the PMA-treated cells (data not shown). Similar studies with the CD45-positive HPB.45.1 cells revealed that PMA induced an increase in CD45 expression and a corresponding increase in PtdIns turnover (data not shown).

To demonstrate more directly that the defect in PtdIns response in the HPB.45.0 is due to its failure to express CD45, we transfected the CD45-negative HPB.45.0 cell line with a murine complementary DNA clone encoding CD45 (ref. 21). The intracellular portions of murine and human CD45, including the tyrosine phosphatase regions, are 85% homologous[22]. Although we have been unable to attain levels of murine CD45 expression comparable to levels of human CD45 observed in the CD45-positive HPB.45.1, several transfected clones do express low levels of murine CD45. Of these, several independent clones have been tested for responsiveness to anti-CD3 mAb. In each case, CD45-positive clones such as HPB.45.0 m45 (Fig. 2*d*; surface staining characteristics shown in Fig. 1*g*, *h*) show a significantly greater increase in $[Ca^{2+}]_i$ than does the CD45-negative HPB.45.0 host (Fig. 2*a*). Thus, even at low levels of expression of murine CD45, substantial restoration of the $[Ca^{2+}]_i$ response was observed.

To begin to localize the site of action of CD45 in regulation of the PtdIns second messenger pathway in human T cells, we examined the function of a heterologous receptor, the human muscarinic receptor type 1 (HM1), in T cells which do not express CD45. This receptor, which is not expressed in human T cells, can activate the PtdIns pathway[15,16,23]. HPB.45.0 was transfected with HM1 and a positive line, HPB.45.0.HM1, was identified by ^{3}H-quinuclidinyl benzylate (a muscarinic antagonist) binding (data not shown). This cell continued to express the TCR and failed to express CD45 (Fig. 1*i*, *j*) The ability of the transfectant to increase $[Ca^{2+}]_i$ in response to stimulation of both HM1 and the TCR was compared with the non-transfected parent and to a Jurkat line transfected with HM1, J-HM1-2.2, which is TCR- and CD45-positive (Fig. 1*k*, *l*). Neither the

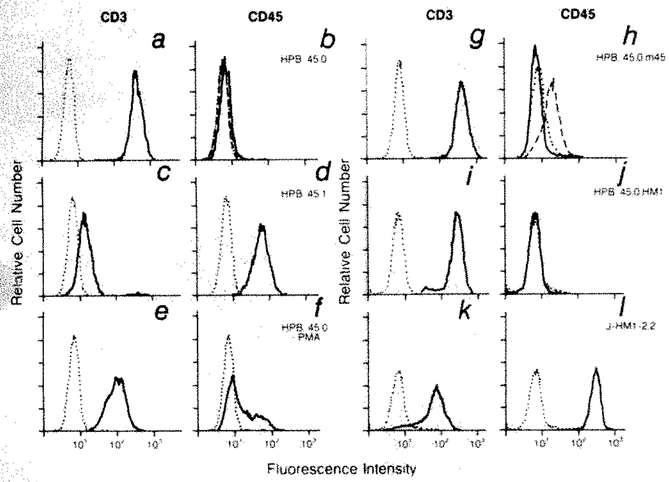

FIG. 1 Surface expression of TCR, human CD45 and murine CD45 on various human T cells. HPB.45.0 (*a*, *b*) and HPB.45.0 · m45 (*g*, *h*) were stained with a control mouse IgG (dotted line); anti-CD3 (Leu4, solid line); anti-human CD45 (HLE, directed against a common determinant of all isoforms of human CD45, solid line); and anti-murine CD45 (M1/9.3.4.HL.2, dashed line). HPB.45.1 (*c*, *d*); HPB.45.0 treated with PMA (Sigma), 50 ng ml^{-1} (48 h; *e*, *f*); HPB.45.0.HM1 (*i*, *j*), and J-HM1-2.2 (*k*, *l*) were stained with control IgG, anti-CD3, and anti-human CD45.

METHODS. Cells were stained as previously described[26]. Antibodies were from Becton-Dickinson except for M1/9.3.4.HL.2 which was from the ATCC, and FITC-conjugated second antibody from Caltag. Analyses were performed on a FACSCAN flow cytometer (Becton-Dickinson) using FACSCAN research software. HPB.45.0 (CD45-negative) and HPB.45.1 (CD45-positive) are clones obtained after limiting dilution of HPB-ALL. HPB.45.0 was transfected with cDNAs for murine CD45 (ref. 21) or HM1 (ref. 16) in expression vectors, by electroporation and selection for G418 resistance as described[16], generating HPB.45.0.m45 and HPB.45.0.HM1. J-HM1-2.2 is a Jurkat derivative expressing HM1 (ref. 16).

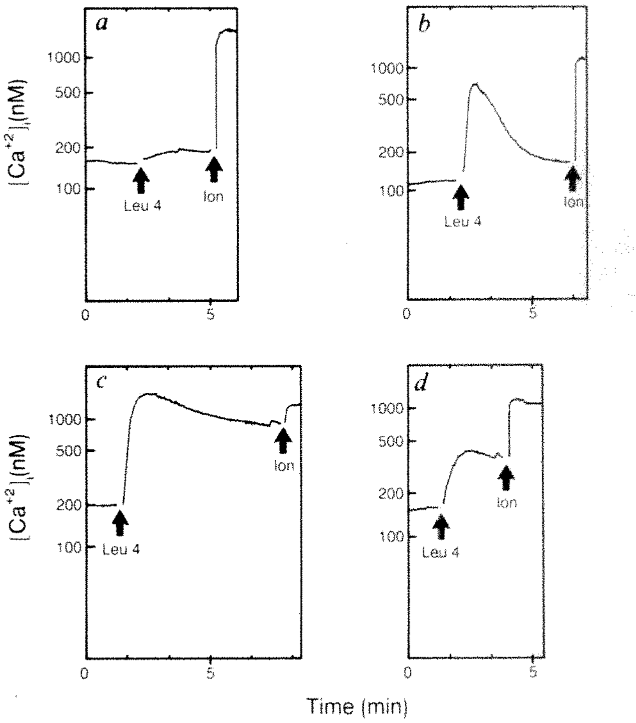

FIG. 2 $[Ca^{+2}]_i$ after stimulation of CD45-negative and CD45-positive derivatives of HPB-ALL. HPB.45.0, CD45-negative (*a*), HPB.45.1, CD45-positive (*b*), HPB.45.0 treated with PMA (50 ng ml^{-1} for 48 h, CD45-positive (*c*), and HPB.45.0.m45, murine CD45-positive (*d*).

METHODS. Each cell type was loaded with 3 μm Indo-1 (Molecular Probes) for 40 min as described[27]. Real time fluorescence was measured at 37 °C with excitation at 334 nm and emission detection at 400 nm in a spectrofluorimeter (Spex Industries). Anti-CD3 mAb (Leu 4) ascites was added (arrow) at a final concentration of 1:1,000 (a saturating amount), ionomycin (Calbiochem) was added at a final concentration of 1 μm. Maximal fluorescence was obtained after lysis of the cells with Triton X-100, minimal fluorescence was obtained after chelation with EGTA. $[Ca^{2+}]_i$ was determined as described[28].

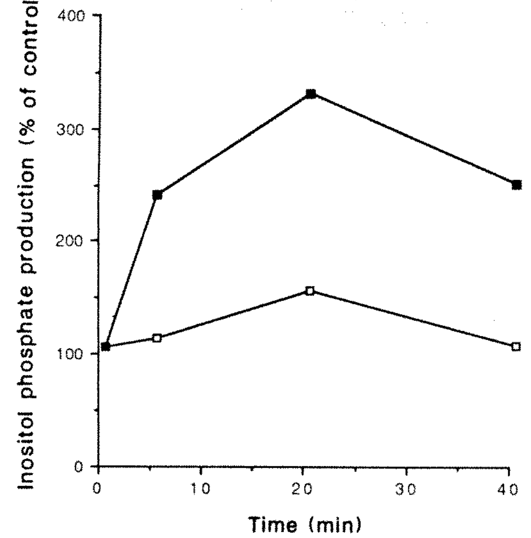

FIG. 3 Time course of soluble inositol phosphate generation in the CD45-negative (□) and CD45-positive (■) cell following TCR stimulation. METHODS. HPB.45.0 (CD45-negative) and HPB.45.1 (CD45-positive) were loaded with [^{3}H]*myo*-inositol (Amersham), 40 μCi ml^{-1} for 3 h in phosphate buffered saline then cultured in RPMI 1640 with 10% FCS (16 h) (ref. 16). The cells were then stimulated with anti-CD3 mAb (Leu 4 ascites, 1:1,000 final concentration) in the presence of 10 mM LiCl. At various time points the cells were lysed and soluble inositol phosphates extracted as described[16]. Data points represent means of duplicates.

muscarinic agonist, carbachol, nor the anti-CD3 mAb elicited a calcium response in the untransfected HPB.45.0 (data not shown). Carbachol induced a brisk calcium increase in J-HM1-2.2, that was reversed with the muscarinic antagonist, atropine. The cells were fully capable of then responding to stimulation via the TCR, as illustrated by the calcium increase seen after addition of mAb (Fig. 4*a*). Figure 4*b* shows that the CD45-negative cell which expresses HM1 also responds to carbachol with a rapid increase in $[Ca^{2+}]_i$, that was antagonized by atropine. Addition of activating mAb to the TCR, however, elicited no increase in $[Ca^{2+}]_i$. Thus, expression of HM1 did not restore TCR-mediated signal transduction function. Assays for the generation of soluble inositol phosphates in these cells resulted in comparable results, such that stimuli inducing $[Ca^{2+}]_i$ increases also elicited increases in inositol phosphates (data not shown).

These data indicate that effective coupling of the TCR to the PtdIns second messenger pathway requires the presence of CD45. The action of CD45 seems to be specific to the TCR activation of the PtdIns second messenger pathway, because in the absence of CD45 the HM1 receptor is fully functional. Although the exact role of CD45 remains to be identified, several studies suggest that this molecule is important in lymphocyte activation[11-14]. Additionally, tyrosine phosphatase activity of CD45 suggests potential interactions with the ζ-chain of the CD3 complex which becomes tyrosine-phosphorylated upon T-cell activation[24] or with Lck, a T-cell-specific tyrosine kinase of the *Src* family whose activity is modified by the presence or absence of CD45 (refs 9, 10). These results provide a potential link between the tyrosine kinase and PtdIns second messenger pathways associated with the TCR in human T cells. Our data, combined with a report of signalling-deficient T cells that are constitutively phosphorylated on CD3 (ref. 25), suggest that CD45 may have an essential role in the control of ζ-chain phosphorylation, which in turn regulates the ability of the TCR to activate the PtdIns pathway in response to stimuli.

Note added in proof: We have recently isolated CD45-negative mutants of the Jurkat T-cell line which are matched for TCR levels when compared with wild-type cells. These CD45-negative cells also fail to increase PtdIns-derived second messengers when stimulated via the TCR. □

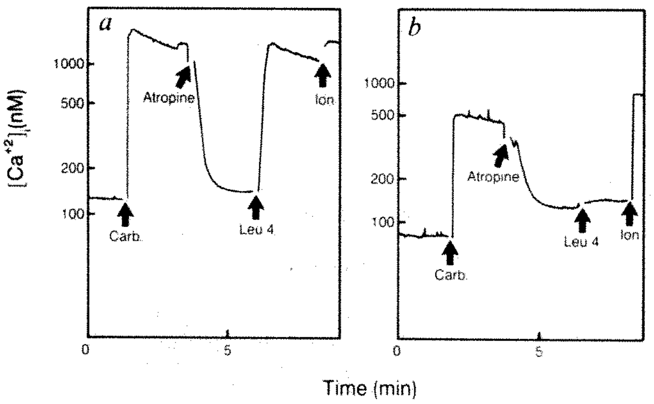

FIG. 4 $[Ca^{+2}]_i$ levels seen after stimulation of the HM1 or TCR in J-HM1-2.2 (*a*) and HPB.45.0.HM1 (*b*.) The assay was performed as in Fig. 2. Carbachol (Sigma) was used at a final concentration of 500 μm and atropine (Sigma) was used at a final concentration of 10 μm.

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Hyperpolarization and relaxation of arterial smooth muscle caused by nitric oxide derived from the endothelium

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STIMULATION of the endothelial lining of arteries with acetylcholine results in the release of a diffusible substance that relaxes and hyperpolarizes the underlying smooth muscle[1–9]. Nitric oxide (NO) has been a candidate for this substance, termed endothelium-derived relaxing factor[10,11]. But there are several observations that argue against the involvement of NO in acetylcholine-induced hyperpolarization. First, exogenous NO has no effect on the membrane potential of canine mesenteric arteries[7]. Second, although haemoglobin (believed to bind and inactivate NO (refs 11–15)) and methylene blue (which prevents the stimulation of guanylate cyclase[11–16]) inhibit relaxation[7,12–14], neither has an effect on hyperpolarization[5,7,8]. Finally, nitroprusside, thought to generate NO in vascular smooth muscle[14,16], relaxes rat aorta without increasing rubidium efflux[17]. Nevertheless, nitrovasodilators, nitroprusside and nitroglycerine cause hyperpolarization in some arteries[18–20]. NO might therefore be responsible for at least part of the hyperpolarization induced by acetylcholine. We now report that hyperpolarization and relaxation evoked by acetylcholine are reduced by N^G-monomethyl-L-arginine, an inhibitor of NO biosynthesis from L-arginine[21–25]. Thus NO derived from the endothelium can cause hyperpolarization of vascular smooth muscle, which might also contribute to relaxation by closing voltage-dependent calcium channels. Our findings raise the possibility that hyperpolarization might be a component of NO signal transduction in neurons or inflammatory cells.

Ring segments of guinea-pig uterine artery were prepared for simultaneous recording of membrane potential and tension development. Phenylephrine (10^{-8}–10^{-5} M) was used to depolarize the membrane and to raise tension (Fig. 1*a*). Infusion of NO caused hyperpolarization and relaxation (Fig. 1*b*). Concentration-response curves to NO-induced (3.6×10^{-8} M–3.6×10^{-5} M) hyperpolarization and relaxation were constructed for segments depolarized by 6 ± 0.5 mV ($n = 25$; Fig. 2). The effector concentration for half-maximum response (EC_{50}) for NO was 1.0×10^{-6} M for the relaxation, and 2.4×10^{-6} M for the hyperpolarization. If, in some arteries, there was a large difference in the doses at which the two responses occurred, this could explain why other studies have reported relaxation in response to NO without detectable hyperpolarization. In uterine arteries, relaxation occurred at the lowest doses of NO without hyperpolarization. At higher levels of depolarization, the maximum relaxation was always accompanied by hyperpolarization, suggesting that the hyperpolarization makes a greater contribution to relaxation when calcium influx is high.

Because NO can evoke hyperpolarization in the uterine artery, we examined whether NO of endothelial origin might account for some of the hyperpolarization in response to acetylcholine. Hyperpolarization and relaxation of uterine arteries in response to acetylcholine were both reduced to a similar extent by N^G-monomethyl-L-arginine, NMMA (2×10^{-5} M, Fig. 3), which reduces endothelium-dependent relaxation in blood vessels[22,23,25]. NMMA significantly reduced hyperpolarization and relaxation of uterine arteries by 41 ± 7% and 50 ± 6%, respectively ($n = 10$; Fig. 3*b*). L-Arginine (10^{-4} M) completely prevented the inhibition caused by NMMA (2×10^{-5} M; Fig. 3*c*). NMMA also reduced acetylcholine-induced hyperpolarization in mesenteric and coronary arteries of guinea-pigs. Thus, endothelium-derived relaxing factor (EDRF) is capable of

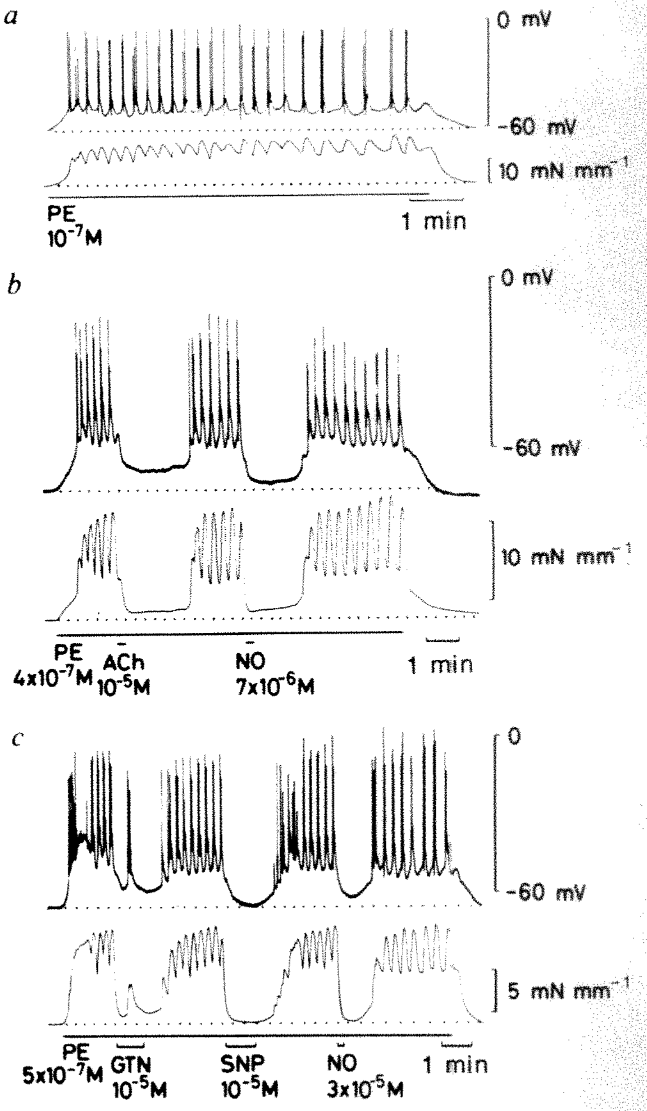

FIG. 1 Acetylcholine, NO and the nitrovasodilators cause hyperpolarization and relaxation of uterine artery. *a*, When the depolarization (upper trace) in response to phenylephrine (PE) was in the range −52 ± 1 mV and −41 ± 1 mV ($n = 22$), oscillations occurred in membrane potential, often topped with action potentials. Each oscillation was followed by a phasic increase in tension (lower trace). *b*, Application of acetylcholine to a vessel depolarized (upper trace) and constricted (lower trace) caused hyperpolarization and relaxation and these responses were mimicked by NO. *c*, Glyceryl trinitrate (GTN) and sodium nitroprusside (SNP) evoked hyperpolarization (upper trace) and relaxation (lower trace) that were similar to those caused by NO.
METHODS. Ring segments (1–2 mm in length) of uterine arteries from guinea-pigs were mounted on a Mulvany–Halpern-style myograph[29] with two stainless steel wires 40 μm in diameter, and continuously perfused with physiological solution containing: NaCl, 120 mM; KCl, 5 mM; KH_2PO_4, 1 mM; $NaHCO_3$, 25 mM; $MgSO_4$, 1.2 mM; $CaCl_2$, 2.5 mM; and glucose, 11 mM; gassed with 95% O_2, 5% CO_2 and maintained at 35 °C. Each segment was stretched in increments until the tension was equivalent to 60–65 mmHg. Membrane potential was measured simultaneously. Solutions of NO were made from NO gas dissolved in deoxygenated water and introduced into the perfusion line with a delay of 1–2 s between the point of injection and the preparation.

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TABLE 1 Effect of membrane potential on hyperpolarization and relaxation in uterine arteries of guinea-pigs

Resting membrane potential (mV)	Membrane potential (mV) in phenylephrine	% Hyperpolarization		% Relaxation	
		ACh (10^{-5} M)	NO (2.5×10^{-5} M)	ACh (10^{-5} M)	NO (2.5×10^{-5} M)
−69 ± 1 (41)	−64 ± 1 (23)	84 ± 10 (13)	114 ± 11 (10)	118 ± 12 (13)	126 ± 5 (10)
	−29 ± 1 (16)	13 ± 5 (9)	27 ± 4 (7)	14 ± 4 (9)	40 ± 8 (7)

Arteries were exposed to phenylephrine (5×10^{-8} to 5×10^{-7} M) and (5×10^{-7} to 5×10^{-5} M) that depolarized the smooth muscle cells to −64 mV and −29 mV, respectively. Hyperpolarization and relaxation are expressed as percentages of the depolarization and constriction, respectively, induced by phenylephrine. Values of *n* are given in parentheses. ACh, acetylcholine.

contributing to this hyperpolarization in these vessels. Because both hyperpolarization and relaxation were not completely abolished, it is possible that another mechanism contributes to the hyperpolarization and relaxation in response to acetylcholine.

Exposure of uterine arteries to methylene blue (10^{-5} M) for 15–20 min before addition of phenylephrine caused depolarization and tension development. In the presence of methylene blue and phenylephrine, the concentration-response curves to NO for both hyperpolarization and relaxation were shifted to the right. After prolonged exposure to methylene blue, both hyperpolarization and relaxation were abolished (Fig. 2*a*, *b*). Both hyperpolarization and relaxation in response to acetylcholine were reduced or abolished after incubation in methylene blue ($n = 5$). Exposure to 10^{-5} M haemoglobin for 10 min before and during application of phenylephrine resulted in abolition of NO-induced hyperpolarization and relaxation (Fig. 2*a*, *b*). Haemoglobin also reduced relaxation in response to acetylcholine in each of seven preparations. In three of these the hyperpolarization was also reduced, but it remained unchanged in the remaining four.

The ability of NO to hyperpolarize uterine arteries might have reflected properties unique to these arteries, but we found that NO hyperpolarized and relaxed all other vessels studied: phenylephrine-depolarized mesenteric ($n = 12$), mammary ($n = 3$) and saphenous ($n = 1$) arteries and aorta ($n = 1$) of guinea-pigs; tail arteries from rats ($n = 3$); saphenous ($n = 1$) and femoral ($n = 1$) arteries from rabbit; mesenteric arteries from dogs ($n = 2$) and guinea-pig coronary arteries at rest ($n = 15$). The ability of some arteries to respond to NO declined with repeated application. That is, there was tachyphylaxis of both the hyperpolarization and relaxation, and the hyperpolarization usually disappeared before the relaxation.

Nitroprusside and nitroglycerine are thought to cause relaxation by liberating NO in smooth muscle cells[14,16]. These nitrovasodilators evoke hyperpolarization in some vascular smooth muscles[18-20] but are without effect on others[17,26-28]. In six uterine arteries depolarized by 7 ± 1 mV and constricted by 1.7 ± 0.3 mN mm^{-1} with phenylephrine (8×10^{-8}–2×10^{-6} M), sodium nitroprusside (10^{-5} M) and nitroglycerine (10^{-5} M) caused hyperpolarization of 138 ± 16% and 110 ± 8%, respectively, accompanied by relaxation of 128 ± 9% and 117 ± 8%, respectively (Fig. 1*c*). In contrast to the actions of acetylcholine, the actions of NO and the nitrovasodilators were independent of the integrity of the endothelium. The nitrovasodilators could hyperpolarize rat tail arteries, but only in those preparations in which the resting membrane potential was more depolarized than −70 mV (ref. 19). NO and acetylcholine could not hyperpolarize uterine arteries that were not depolarized by phenylephrine. These arteries have a high negative resting membrane potential (−69 ± 1 mV, $n = 41$), compared with mesenteric and coronary arteries (−61 ± 2 mV ($n = 12$) and −58 ± 2 mV ($n = 15$), respectively), and in none of these arteries could acetylcholine or NO hyperpolarize the membrane beyond about −70 mV.

The hyperpolarization in response to acetylcholine is due to an increase in potassium conductance[2,3,8]. When the potassium concentration in the perfusing solution was raised (10–100 mM), the hyperpolarization induced by both acetylcholine and NO in uterine arteries was reduced or abolished. Others have shown that the ability of acetylcholine to induce hyperpolarization and relaxation is dependent on the extent of depolarization elicited by the raised external potassium. At large depolarizations, there is a reduction in amplitude of both the hyperpolarization[2,8] and the relaxation[17]. It can be seen from Table 1 that the hyperpolarization and relaxation induced by acetylcholine (10^{-5} M) and NO (2.5×10^{-5} M) are also dependent on the extent of depolarization induced by phenylephrine in uterine arteries. Thus, there is a range of membrane potential in which NO can

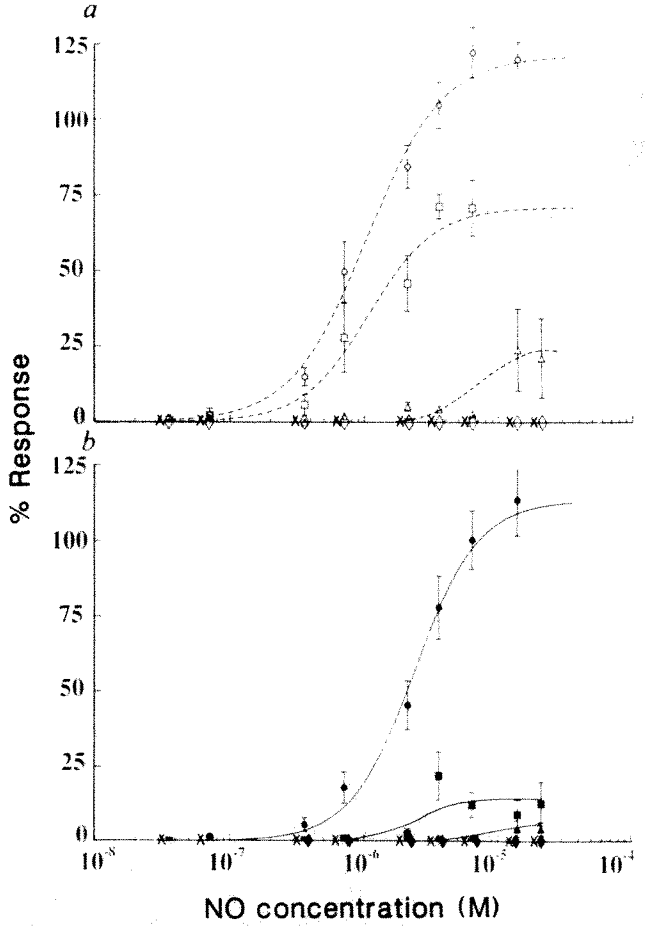

FIG. 2 Dose-dependent hyperpolarization of the membrane and relaxation evoked by NO in uterine arteries of guinea-pigs. *a*, Relaxation (open symbols). *b*, Hyperpolarization (filled symbols). The arteries were exposed to concentrations of phenylephrine (10^{-8}–5×10^{-6} M) that depolarized the smooth muscle by 6 ± 0.5 mV and raised tension by 4 ± 0.4 mN mm^{-1}. Concentrations of NO (4×10^{-8}–4×10^{-5} M) were applied and the hyperpolarization and relaxation that resulted expressed as a percentage of the phenylephrine-induced depolarization and increase in tension, respectively. NO evoked concentration-dependent relaxation (*a*) and hyperpolarization (*b*) in uterine arteries (circles; $n = 24$). Methylene blue (10^{-5} M) caused time-dependent inhibition of both relaxation ($n = 10$) and hyperpolarization ($n = 9$; squares, 21 min; triangles, 53 min; diamonds, 81 min). Both responses were abolished after 81 min. Exposure to haemoglobin (10^{-5} M) abolished the responses (crosses; $n = 7$). Vertical bars represent standard errors of the mean. The number of animals studied (*n*) (not the number of cells impaled) is used throughout.

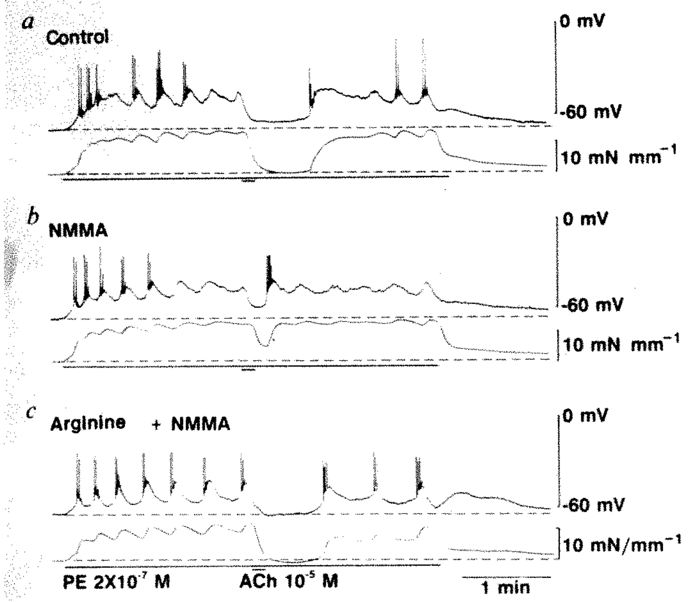

FIG. 3 Inhibition of acetylcholine-induced hyperpolarization and relaxation by L-NMMA. *a*, Hyperpolarization (upper trace) and relaxation (lower trace) evoked by 10^{-5} M acetylcholine in a uterine artery depolarized and constricted with phenylephrine (PE). *b*, Preincubation of the tissues with NMMA acetate (2×10^{-5} M) reduced the amplitude and duration of the hyperpolarization and relaxation in response to 10^{-5} M acetylcholine. *c*, The effect of this concentration of NMMA was antagonized by 10^{-4} M L-arginine, added during and for 10 min before application of NMMA.

cause hyperpolarization. The more negative potentials are close to the equilibrium potential for potassium, whereas at substantial levels of depolarization, the magnitude of total conductance swamps NO-induced potassium conductances. The level of membrane potential in determining the ability of acetylcholine and NO to elicit hyperpolarization may explain why others have failed to find an effect of NO on membrane potential. Moreover, in our study membrane potential and developed tension have been measured simultaneously in the same tissue.

In conclusion, NO, whether derived from the endothelium as EDRF or from nitrovasodilator drugs, can mediate hyperpolarization of arterial smooth muscle. Although NO can induce relaxation by stimulation of calcium extrusion alone, the contribution of the hyperpolarization to relaxation is probably more important when calcium influx is high. ☐

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Subsecond deactivation of transducin by endogenous GTP hydrolysis

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THE response of a retinal rod cell to a weak flash of light is mediated by a receptor/GTP-binding protein (rhodopsin/transducin) signal transduction system[1,2] and terminates within a second[3]. The Tα subunit of transducin (composed of subunits Tα, Tβ and Tγ) is triggered by photoexcited rhodopsin (R*) to release GDP and bind GTP. The binding of GTP causes release of the Tα unit from Tβγ and allows it to modulate the activity of an enzyme that generates a second messenger. Termination of the response requires the hydrolysis of the GTP by intrinsic GTPase[4,5]. As with other G proteins, the GTPase activity of transducin seems to be slow. Reported *in vitro* turnover rates of a few molecules of GTP hydrolysed per molecule of transducin per minute[6–9] imply a Tα-GTP deactivation time of many seconds. But this time might be only a small fraction of that of the GTPase cycle. We have now used time-resolved microcalorimetry[10–12] in bovine rod outer segments (ROS) to monitor the heat release due to the hydrolysis of GTP by a transducin population that had been quickly activated by flash illumination of rhodopsin. The enthalpy of GTP hydrolysis is released within 1 s at 23 °C. This deactivation time seems to be independent of any diffusible factor in the preparation and concurs with the termination kinetics of the rod's response. Thereafter, transducin seems unable to reload GTP for many seconds. This refractory 'resetting' time may account for the low steady-state GTPase rates *in vitro*.

In vitro steady-state measurements of the GTPase rate of transducin under continuous illumination (a condition for receptor saturation) assay a complete cycle that includes[13] encounter of Tα-GDP-Tβγ with photoexcited rhodopsin, R*-catalysed GDP/GTP exchange on Tα, dissociation of active Tα-GTP from R* and Tβγ, deactivation by endogenous GTP hydrolysis, and reassociation of Tα-GDP with Tβγ before a new cycle can begin. The first three steps determine the millisecond activation time of transducin[14], whereas the hydrolytic step determines the deactivation time. Any other steps such as a long-lived post-hydrolytic GDP-Pi conformation (as with tubulin or actin) or a slow re-association of Tα-GDP with Tβγ will lengthen the GTPase cycle.

Whereas steady-state techniques necessarily give a sum total of these times, they may be separated by a time-resolved technique applied to a transiently activated sample.

Under weak flash illumination, fast quenching of R* is achieved *in vivo* by the combined action of rhodopsin kinase and arrestin[15], the latter taking <0.2 s to bind to phosphorylated R* (ref. 16). *In vitro*, this quenching takes seconds even with kinase and arrestin enrichment of concentrated ROS suspensions[15,17]. If needed, however, hydroxylamine can be used to accelerate the deactivation of R* by displacing its retinal[18].

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Most of the enthalpy of GTP hydrolysis is released in three steps: R*-catalysed GTP loading and associated transconformation of Tα; hydrolysis of Tα-GTP to Tα-GDP per se; and reassociation of Tα-GDP with T$\beta\gamma$. So the kinetics of this enthalpy release constitutes an upper-bound of the Tα-GTP lifetime. We assessed the enthalpy release using a custom-designed time-resolved microcalorimeter (Fig. 1), on flash-illuminated ROS suspensions. With the addition of both GTP and cyclic GMP (Fig. 2*a*) there arises a large signal resulting mainly from the amplified hydrolysis of cGMP by the activated cGMP phosphodiesterase (cGMP-PDE). Hydrolysis of known amounts of cGMP provides an internal calibration of the cell's thermal sensitivity (Fig. 2*b*). With the addition of GTP only, a smaller signal due to light-induced GTP hydrolysis (Fig. 3*a*) is observed. It follows a fast spike due to heating by the absorbed light flash that is also seen alone in the absence of GTP or after its exhaustion (Fig. 3*c*). The signal with only GTP present is not due to contaminant cGMP as the preparations used have in the dark a nonspecific PDE[19] that is more active than the guanylate cyclase (Fig. 2*b*).

The GTP hydrolysis signal almost saturates at $R^*/R = 2.6 \times 10^{-4}$ (Fig. 3*b*), where the integrated amplitude of the signal roughly corresponds to one turnover of the transducin pool (using a ΔH value of 5-6 kcal mol^{-1} for GTP hydrolysis, based on the ΔG value of 7.3 kcal mol^{-1} for ATP hydrolysis). At low levels of NH_2OH this hydrolysis signal displays a fast pulse component and a slow long tail (Fig. 3*a*, *g*). The rise time of the pulse component is about 0.3 s, its fall time about 0.6 s. With only 5 mM NH_2OH, R* lasts 6-10 s[18] during which it continuously catalyses the turnover of transducin. The pulse component in Fig. 3*a* thus corresponds to the first round of GTP hydrolysis by all the transducin that is ready before the light flash: immediately after illumination and once only, transducin is quickly activated and almost synchronously deactivated by GTP hydrolysis. Subsequently, and as long as some R* persists, transducin turns over. The rate of the heat release is then governed by the duration of the whole cycle which must include one or many slow steps as the long tail in Fig. 3*a* is much smaller than the pulse. Thus, if there is little NH_2OH, and some R* persists, transducin will recycle but the GTPase turnover rate will be limited by the slow 'resetting' steps. One might postulate a long-lived intermediate refractory Tα (Tα_{ref}) that still contains the γ-phosphate and/or is still soluble like Tα-GTP and thus cannot reassociate with T$\beta\gamma$. With 25 mM NH_2OH (Fig. 3*d*), one expects R* to decay faster, resulting in little or no transducin turnover and a concomitant reduction or disappearance of the tail component, which is indeed the case. As the R* lifetime should not kinetically influence transducin activation and

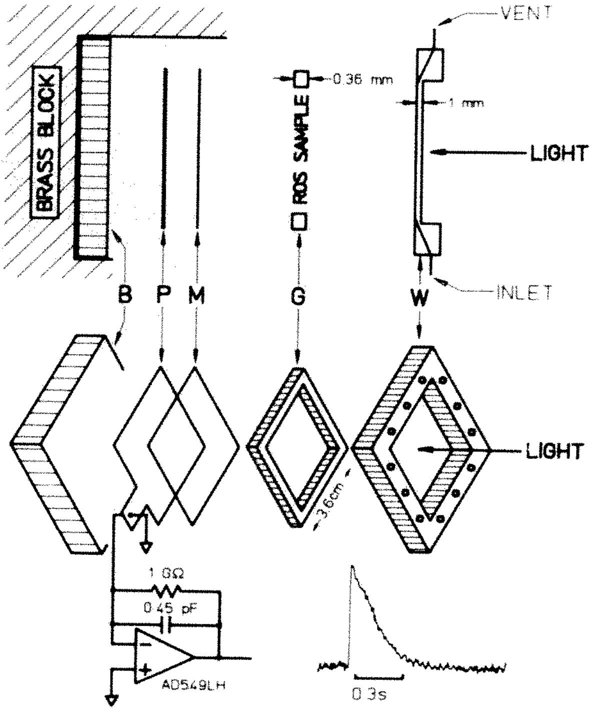

FIG. 1 The active element of the microcalorimeter is a 40-μm-thick poled, polyvinylidenefluoride (PVDF)[25] film gold-plated on both faces (P) (Solvay & Cie, Bruxelles). The back electrode is epoxy-glued to a thermally insulating slab (10-mm thick) of balsa wood (B) and the front electrode is protected from the liquid sample by a 6-μm mylar layer (M). A 0.36-mm-thick Parafilm gasket (G) and a Teflon window (W) define a 3.6 × 3.6-cm sample chamber. The film's grounded front electrode acts as a shield to the back electrode which is connected to the operational amplifier. The cell thus arranged is adiabatic. It is mounted together with the amplifier between two 15-kg blocks of brass (S) stabilized at 23 °C in a styrofoam box; the block in front has a double-paned window through which the sample can be illuminated. This instrument is an improved version of one described before[11]. Dilation from heating causes the PVDF polarizability to decrease, generating a displacement current proportional to the derivative of the film temperature[25]. The integral of this signal is proportional to the quantity of heat received by the film. This device thus monitors in real time the rate of reactions through their enthalpy release. The trace shown is the instrument's response obtained by flashing a dye solution. Direct heating of the PVDF by the light flash was accounted for by subtraction using the same procedure as in ref. 12.

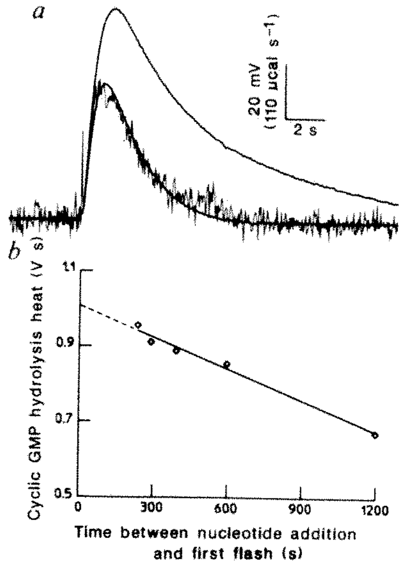

FIG. 2 Cyclic GMP hydrolysis signals and microcalorimeter calibration. *a*, Flash-induced cGMP hydrolysis in dilute ROS samples: 3.8 μm rhodopsin, 0.2 mM GTP, 3 mM cGMP, 0.5 mM NH_2OH (upper trace), 50 mM NH_2OH (lower thick trace) or 200 mM NH_2OH (lower thin trace, signal ×14 to normalize to that with 50 mM NH_2OH), flash photoexcitation $R^*/R = 5.2 \times 10^{-5}$. *b*, Calibration by cGMP hydrolysis in dilute samples (composition and photoexcitation as in *a*, lower thick trace). As there is a nonspecific PDE activity in the dark[19], samples were illuminated at different times after nucleotide addition by series of 20 flashes to exhaust the cGMP (GTP hydrolysis heat is $<10^{-3}$ times that of cGMP hydrolysis); the total hydrolysis heat from each sample is the sum of the integrated areas of these 20 signals. Using $\Delta H = 12.3$ kcal mol^{-1} (ref. 12) for cGMP hydrolysis, zero-time extrapolation of the linear fit through these sums gives a calibration value (1 mV.s = 5.5 μcal) about equal to that obtained from flash-heating a dye solution.

METHODS. Bovine ROS prepared as in ref. 26 and stored at −70 °C were resuspended into buffer in total darkness and incubated 20 min at 23 °C with 1 mM NH_2OH to scrub R* formed before or during preparation. This concentrated (150–250 μM rhodopsin) stock suspension was stored on ice until use. Adequate amounts of buffer, GTP, cGMP, NH_2OH (adjusted with KOH to pH 7.5), were added and the sample (0.46 ml) injected into the cell. Measurements were begun after 4–5 min to allow thermal equilibration with the cell. Buffer composition: 100 mM KCl, 20 mM HEPES, pH 7.5, 2 mM $MgCl_2$, 0.4 mM DTT.

deactivation, the width of the pulse in Fig. 3*d* is mostly identical to that of Fig. 3*a*. At still higher NH_2OH levels (50 mM, data not shown), R* is too short-lived to activate the whole transducin pool: the GTP hydrolysis pulse slightly decreases in amplitude while maintaining the same kinetics. This effect is also seen with the cGMP hydrolysis signal: with an increase in NH_2OH concentration from 0.5 to 50 mM, a long tail disappears, whereas with a change of 50 to 200 mM NH_2OH, signal amplitude decreases but there is no change in kinetics (Fig. 2*a*).

The kinetics of the GTP hydrolysis pulse is unchanged by a two fold dilution of the sample (Fig. 3*e*) and therefore seems independent of a soluble accelerating cofactor.

Double-flash experiments reveal a refractory time during which the GTP signal cannot be fully re-elicited. Recovery takes 4–8 s at 50 mM NH_2OH (Fig. 4*a–c*), more than 12 s at 10 mM NH_2OH (Fig. 4*d–e*) and 2 min at 2 mM NH_2OH (not shown). A high NH_2OH concentration quickly deactivates R*, and transducin activation ends soon after the flash. The 4–8-s refractory time is then the resetting time needed to replenish the Tα-GDP-Tβγ pool before another signal is possible. With little NH_2OH, R* and transducin activation persist after the flash: turnover occurs, governed by the slow resetting process. Hence, $T\alpha_{\text{ref}}$ and/or Tα-GDP accumulate, but the Tα-GDP-Tβγ pool is hardly replenished: a second flash elicits no signal (Fig. 4*d*); the longer refractory time at low NH_2OH concentration merely approximates the R* lifetime during which Tα-GDP-Tβγ is not replenished.

The R*-quenching effect of NH_2OH is not strictly required in demonstrating the fast kinetics of endogenous GTP hydrolysis, as the activation-deactivation times are much faster than the resetting time. This significant kinetic difference allows the separation of the two processes even when there is transducin turnover at low NH_2OH concentrations (Fig. 3*a*). High NH_2OH concentrations, when used in less crucial experiments, seem relatively harmless. As checked by the pH metric technique[20] on continuously illuminated samples, the saturated cGMP-PDE activity is not modified by addition of 50 mM NH_2OH (data not shown). Furthermore, hydroxylamine does not seem to accelerate transducin deactivation, as the GTP hydrolysis pulse does not speed up when the concentration of NH_2OH increases from 5 mM to 25 mM (Fig. 3*a*, *d*).

The *in vitro* kinetics of the transducin GTPase cycle thus include three or four steps: (1) activation, Tα-GDP-Tβγ → Tα-GTP + Tβγ, taking about 0.1 s; (2) deactivation, Tα-GTP → $T\alpha_{\text{ref}}$, completed within 1 s; (3) regeneration, $T\alpha_{\text{ref}}$ → Tα-GDP; and/or (4) reassociation: Tα-GDP + Tβγ → Tα-GDP-Tβγ, which may last many seconds.

Being in the subsecond range, endogenous GTP hydrolysis qualifies kinetically as a shut-off mechanism for visual transduction; its speed does not depend on soluble cofactors (contrary to refs 21 and 22), but transducin interaction with native disk membranes and, as suggested for other G proteins, a physiological ionic composition[23], might be crucial. The resetting time during which transducin cannot reload GTP accounts for the reported low steady-state GTPase rates. This could be an *in vitro* effect due partially to the solubilization of Tα-GTP causing a slow recombination of the resulting Tα-GDP to membrane-bound Tβγ. Although Gα-GTP subunits of other G proteins do not seem as soluble as Tα-GTP, their reported GTPase rates *in vitro* might also be poor indicators of their lifetimes *in vivo*

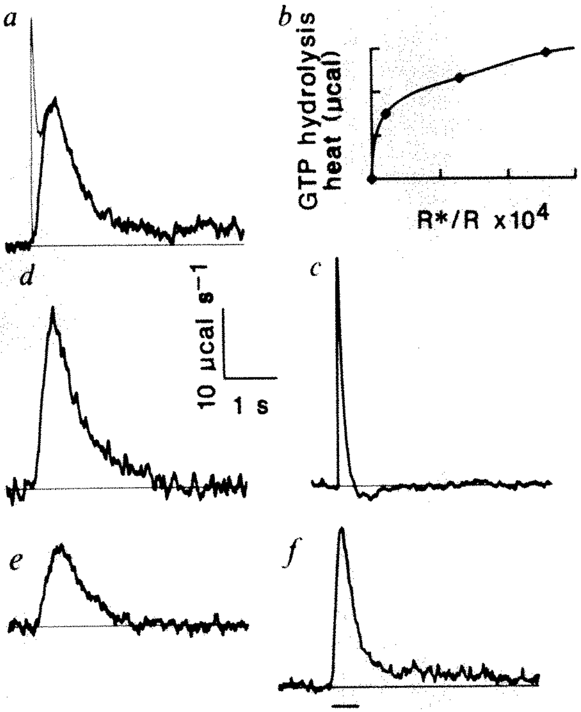

FIG. 3 Kinetics of GTP hydrolysis. *a*, Light-induced GTP hydrolysis signal at low NH_2OH: 63 µM rhodopsin, 2 mM GTP, 5 mM NH_2OH, $R^*/R = 1.3 \times 10^{-4}$ (thin trace). The spike preceding the broader signal is due to direct heating of the PVDF film by the light flash. *b*, Integrated areas (in µcal) of GTP hydrolysis signals as a function of photolysis levels: 75 µM rhodopsin, 2 mM GTP, 50 mM NH_2OH, $R^*/R = 2.2 \times 10^{-5}$, 1.3×10^{-4} and 2.6×10^{-4}. *c*, Average of eight flash spikes collected after GTP exhaustion; its subtraction from the raw signal reveals the heat generated by the sample itself (*a*, thick trace). The slight negative component is due to cooling of the PVDF film by the exhausted sample. *d*, *e*, GTP hydrolysis signals at higher NH_2OH from: 75 µM (*d*), 33 µM (*e*) rhodopsin, 2 mM GTP, 25 mM NH_2OH, $R^*/R = 1.3 \times 10^{-4}$. *f*, Average of two experiments at low NH_2OH levels (same composition and illumination as in *a*) shown on a ×2 compressed time scale. These GTP hydrolysis signals are repeatable up to 50 times. The signals in *a*, *d*, *e* and *f* come from samples of the same preparation. (The significant light-independent GTPase activity of these ROS samples adds only a constant to the baseline.)

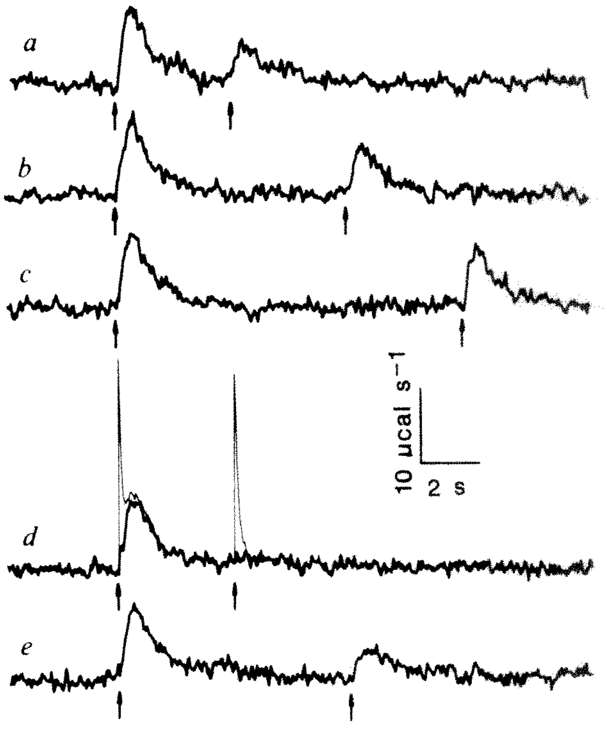

FIG. 4 Double-flash experiments. Varyingly spaced pairs of flashes (arrows, $R^*/R = 1.3 \times 10^{-4}$) from two aliquots containing 69 µM rhodopsin, 2 mM GTP, with 50 mM NH_2OH (*a–c*), or 10 mM NH_2OH (*d*, *e*). Each trace is an average of four (*a–c*) or two (*d*, *e*) recordings separated by 1–2 min. The flash spikes have been subtracted (as in Fig. 3*a*) except in *d* (thin trace) where both flash spikes can be seen in the unsubtracted data.

which control the duration of effector activation, and hence the integrated intensity of cellular responses. The recent demonstration that a mutated Gα with reduced GTPase activity is involved in cellular transformation[24] stresses the importance of an accurate knowledge of the true lifetime of Gα-GTP. ☐

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Development of venous occlusions in mice transgenic for the plasminogen activator inhibitor-1 gene

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THE fibrinolytic potential of the vasculature is modulated primarily by the availability and activity of plasminogen activators, which convert the zymogen plasminogen into the active fibrin-degrading enzyme plasmin[1]. The activities of these key regulatory enzymes are directly neutralized by their primary endogenous inhibitor, plasminogen activator inhibitor-1 (PAI-1)[2–6]. Although some individuals with a tendency to develop thrombotic disorders exhibit elevated levels of PAI-1 in their plasma[7–10], the cause-and-effect relationship between increased PAI-1 and thrombosis is still unclear. Specifically, it is not known whether chronic depression of fibrinolytic activity results in the development of thrombosis. To address this question we developed transgenic mice in which the contribution of PAI-1 to thrombus formation could be evaluated. The results presented in this report indicate that elevated levels of PAI-1 contribute to the development of venous but not arterial occlusions.

The DNA construct used in the production of PAI-1 transgenic mice contained the murine metallothionein I promoter, human endothelial cell PAI-1 complementary DNA, and the bovine growth hormone polyadenylation signal sequence. Mice putatively transgenic for human PAI-1 showed a single, early indication of disturbance of the haemostatic system (Fig. 1*a* and *b*). A distinct area of subcutaneous haemorrhage was apparent at the tip of the tails of mice at about 3 days after birth. This change became increasingly severe as the mice aged, until by ~12 days after birth the tip of the tail was necrotic and, in addition, the mice had swollen hind feet (Fig. 1*c*, right). At this age, no other changes were noted. Putative nontransgenic litter mates had normal tails and hind feet (Fig. 1*c*, left). The necrotic tails and swollen hind feet seemed to be the result of venous occlusions detected hisotologically (Fig. 1*e* and *f*). These occlusions were seen in pups as young as 3 days (the earliest histological examination) and were localized exclusively in the venous circulation; the arteries of the distal tail and limbs were patent. This localization is probably related to the observed haemorrhage and oedema. The thrombi consisted predominantly of mononuclear leukocytes, but also contained platelets, erythrocytes and fibrin. The presence of muscle degeneration and inflammation in 3-day-old mice indicates very early onset of the occlusive disorder, and may at least partially explain why cellular, as opposed to simply fibrin-platelet, thrombi were primarily detected. By ~2 weeks of age, the necrotic portion of the tails had completely sloughed off, whereas the swollen hind limbs appeared normal. This resolution of the occlusions in, and recovery of, the hind limbs of the mice may have been caused by compensatory mechanisms (for example, increased plasminogen activator expression) or by a decline in PAI-1 transgene expression. Therefore, the relationship between the time-dependent existence of the lesions and the levels of PAI-1 DNA, messenger RNA and protein was examined.

DNA extracted from the tails of mice displaying the circulatory disturbances described hybridized with a radiolabelled human PAI-1 cDNA probe, whereas DNA obtained from litter mates having normal tails or from control mice showed little or no hybridization (Fig. 2). Based on these results, 13 founder (G_0) animals were identified, having gene copy numbers of 1-10. In all instances, the existence of the venous occlusions correlated with the presence of the PAI-1 transgene. Assays for PAI-1 protein, activity, and mRNA were then used to correlate PAI-1

TABLE 1 PA1-1 DNA, mRNA and protein in tissues of transgenic mice

Phenotype (*a*)	Tissue					
	Spleen	Liver	Kidney	Heart	Brain	Lung
Control	ND	6.1	ND	0.8	0.1	0.1
Normal tail	5.8	6.9	8.0	1.1	1.1	0.2
Short tail	438.2	176.3	126.5	12.7	12.2	7.0

(*b*)	DNA			mRNA			Protein		
Age (days):	4	7	28	4	7	28	4	7	28
Control	0	0	0	<0.5	<0.5	<0.5	10	4	6
Normal tail	4	2	2	1.4	2.5	<0.5	11	10	9
Short tail	11	8	8	5.6	3.5	<0.5	210	98	21

a, Levels of PAI-1 protein in tissues obtained from 4-day-old control and G_1 and G_2 mice of differing phenotypes. Values are expressed as nanograms of PAI-1 per g wet weight of tissue. ND, not detectable. *b*, PAI-1 DNA copy number and levels of PAI-1 mRNA and protein in samples of liver tissue obtained from control and G_1 and G_2 transgenic mice of various ages. Messenger RNA values are expressed as pg per μg of total RNA and protein values are expressed as ng per g wet weight of tissue. Three to five animals were analysed in each group. DNA was prepared as described in Fig. 2. The DNA samples were then assayed by Southern blot analysis in which 10 μg was digested with restriction enzymes, electrophoresed in a 1.0% agarose gel, blotted onto nitrocellulose, and probed with radiolabelled PAI-1 cDNA. PAI-1 gene copy-number of the transgenic mice was determined using standard amounts of the injected transgene in parallel lanes of a Southern blot of the transgenic mouse genomic DNA. RNA was prepared as described in ref. 16 and measured spectrophotometrically. RNA preparations (10 μg) were denatured in 12 × SSC and 15% formaldehyde at 60 °C for 15 min. The denatured RNA was applied to nitrocellulose using a slot blot apparatus (Schleicher & Schuell) and baked at 80 °C *in vacuo*. The RNA samples were then probed with radiolabelled PAI-1 cDNA, and PAI-1 mRNA levels were determined by comparison to a PAI-1 mRNA standard curve generated from the PAI-1 cDNA in pBS using the T3 promoter. For PAI-1 protein determinations, tissues were weighed and then extracted in buffer containing 0.1% Triton X-100. After centrifugation of the extracts, the supernatant fluids were analysed for PAI-1 protein using a monoclonal antibody enzyme-linked immunosorbent assay (American Diagnostica).

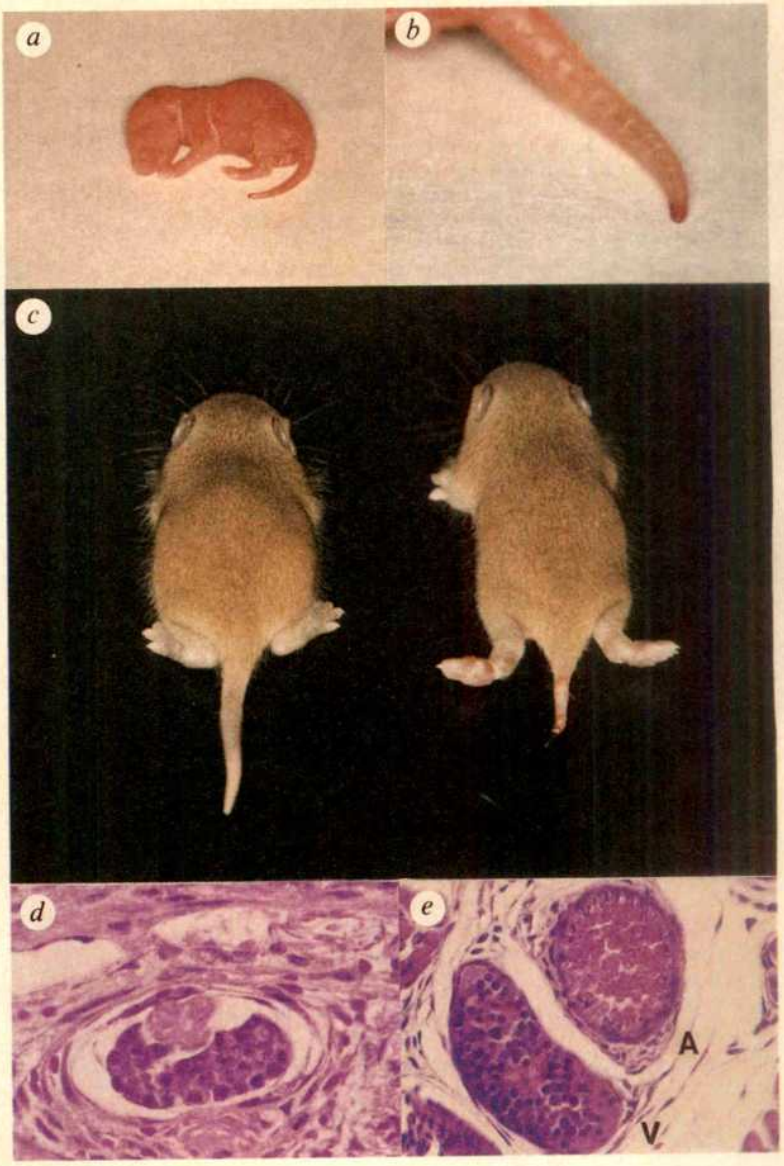

FIG. 1 Morphology and histological analysis of PAI-1 transgenic mice. *a*, Putative PAI-1 transgenic mouse photographed 3 days after birth. *b*, Higher magnification view of the tail region shown in *a*. *c*, Mice photographed 12 days after birth. Left, mouse with normal tail and hind feet; right, transgenic mouse showing necrosis and sloughing of the tail and swollen hind feet. *d*, Histological cross section through the tail vein of 7-day-old PAI-1 transgenic mouse with necrosis of the tail. Note that the cellular thrombus appears to be surrounding a valve leaflet. *e*, Section through the swollen hind foot of a PAI-1 transgenic mouse. Note that the vein (V) appears to be completely occluded, whereas the artery (A) appears normal.

METHODS. The transgene construct for PAI-1 was assembled as a four-part ligation. A *Bam*HI fragment (1,397 base pairs (bp)) was isolated from a PAI-1 plasmid containing the complete coding region, 80 bases of the 5′ untranslated region, and 140 bases of the 3′ untranslated sequence (obtained from human umbilical vein endothelial cells). The PAI fragment was ligated on the 5′ end to a 1,800-bp *Eco*RI–*Bgl*II metallothionein promoter fragment derived from pdBPV-MMT neo (342-12) (obtained from P. Howley, NIH) and on the 3′ end to a 220-bp *Bam*HI–*Sph*I fragment from the polyadenylation signal sequence of bGH (obtained from F. Rottman, Case Western). The vector was pBS (Bluescribe; Stratagene) prepared as an *Eco*RI–*Sph*I fragment. The metallothionein promoter and the bGH polyadenylation signal DNA were phosphatased and ligated to the PAI DNA overnight 12 °C at a 2:1 molar ratio. In collaboration with DNX (Athens, Ohio) eggs from B6/SJL-F_1J mice were microinjected with the ~3,400-bp *Eco*RI–*Sph*I DNA fragment and implanted in foster mothers according to established protocols. Mice were photographed with a Nikkormat camera using a medical lens and Kodak 100 ASA, daylight-balanced film. For histological evaluation, 30 G_1 and G_2 mice were killed at various ages by CO_2 inhalation. Tissues were fixed by immersion in 10% neutral-buffered formalin, processed, and embedded in paraffin according to established procedures. Tissue cross sections (5 μm) were cut, stained with haemotoxylin and eosin, and examined by light microscopy.

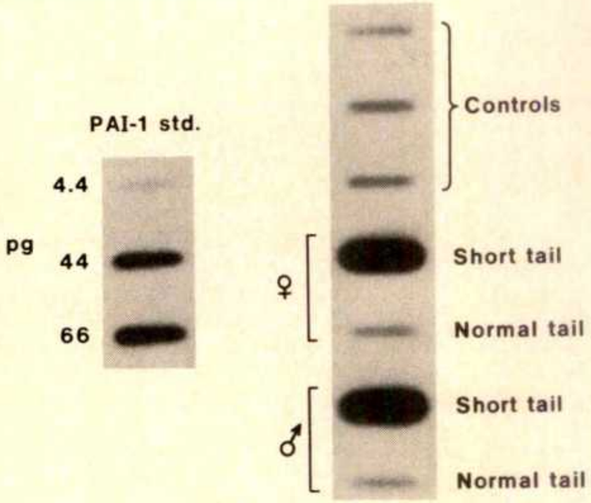

FIG. 2 PAI-1 DNA in tails of adult transgenic mice. Left, PAI DNA standard (std.); top right, tail DNA obained from control mice; bottom right, tail DNA from female and male short-tailed and normal-tailed mice.

METHODS. DNA was prepared from tails by dicing them with a razor blade into 700 μl of SSTE buffer (100 mM NaCl, 1% SDS, 50 mM Tris buffer and 15 mM EDTA, pH 8.0), then digestion with 0.5 $\mu g\ ml^{-1}$ proteinase K at 55 °C for 12 h. The mixture was then treated with 0.2 $\mu g\ \mu l^{-1}$ RNase at 37 °C for 1 h. An equal volume of phenol was added and the mixture was extracted twice. The aqueous phase was extracted with chloroform–isoamyl alcohol (24:1) and precipitated with isopropanol. The DNA was pelleted and washed with 70% ethanol and dried under vacuum. Buffer (100 μl TE) was added to each sample and the DNA quantitated spectrophotometrically. For slot blots, 10 μg purified DNA was fixed onto nitrocellulose and probed with a radiolabelled PAI-1 cDNA.

expression with the morphological and hisological profiles of the mice. Of the thirteen G_0 mice, six were selected for generation of the G_1 and G_2 lines used for subsequent analyses. Table 1(*a*) clearly indicates that tissue levels of PAI-1 protein also correlated directly with the presence of occlusions. Presumably, baseline PAI-1 detected in control and normal-tail animals reflects a low level of cross-reactivity between the anti-human PAI-1 monoclonal antibodies and endogenous murine PAI-1. Increased amounts of PAI-1 in the liver and kidney were expected on the basis of previous studies using the metallothionein promoter[11]; however, the raised level of PAI-1 protein detected in spleen tissue obtained from transgenic animals was surprising, and is as yet unexplained. The PAI-1 content of the cellular occlusions was not examined. These data show a strong link between the existence of venous occlusions and the presence and expression of the PAI-1 transgene. This relationship was examined more closely by determining the PAI-1 DNA copy-number and by measuring the PAI-1 mRNA and protein levels

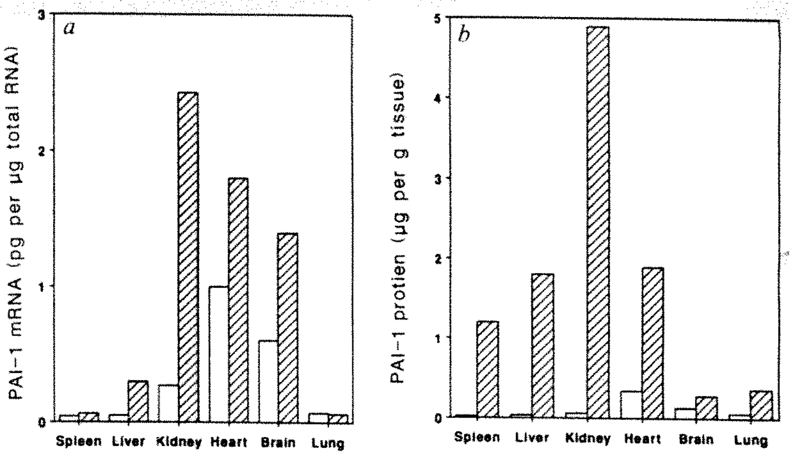

FIG. 3 Induction of PAI-1 expression in 28-day-old transgenic mice with ZnSO$_4$. *a*, PAI-1 mRNA in tissues of transgenic mice. *b*, PAI-1 protein in tissues of transgenic mice. Open bars, PBS control; hatched bars, ZnSO$_4$-injected. PBS or PBS containing ZnSO$_4$ was administered to 28-day-old mice IP (10 mg kg^{-1}). Eight hours after injection, mice were killed by cervical dislocation and tissues removed for RNA and protein analyses as described in Table 1.

in the livers of mice killed at different times after birth (Table 1(*b*)). As expected, DNA copy-number did not vary significantly within a phenotype. Both PAI-1 mRNA and protein declined, however, in the transgenic ('short tail') mice 4–28 days after birth to levels detected in control and normal-tail (non-expressing transgenic) mice. This decline paralleled the resolution of the venous occlusions in the hind limbs, and provides additional evidence of a strict correlation between the presence and expression of the PAI-1 transgene and the development of occlusions.

The decline of PAI-1 expression during the first 4 weeks after birth could result from the loss of responsiveness of the metallothionein promoter to endogenous control mechanisms. We addressed this issue by administering ZnSO$_4$ to 28-day-old transgenic mice that had shown tail necrosis and swollen hind limbs earlier in life. This treatment induced the expression of the PAI-1 gene from baseline levels to levels that in certain tissues exceeded those observed at day 4 (Fig. 3). Plasma PAI-1 was increased by ~20-fold after ZnSO$_4$ treatment (data not shown). We conclude from these experiments that the metallothionein promoter was intact and responsive. Studies of the mouse[12] and the chick[13] have shown that expression of endogenous metallothionein peaks shortly before birth (mouse) or after birth (chick) and declines thereafter. Taken together, these data indicate that transgenic PAI-1 may decline after day 4 as a consequence of the normal function of mechanisms governing the expression of endogenous metallothionein.

We found that the level of PAI-1 protein directly reflects the amount of PA neutralizing activity detectable (data not shown). In addition, the occlusions in the PAI-1 transgenic mice are reminiscent of those present in protein C-deficient patients in that they are localized to the venous side of the circulation[14]. Thus, it is conceivable that neutralization of endogenous murine activated protein C may also contribute to the observed venous occlusive disorder. Distinguishing between these and other possibilities is made particularly difficult by the fact that the critical period of evaluation is before 4 days after birth. Indeed, it has not been possible to measure the endogenous circulating levels of these murine pro-fibrinolytic factors because of the small amounts of blood obtainable from mice of this age. Alternatively, the occlusions may result from a perturbation of the vascular endothelium that is associated with chronically elevated PAI-1. In this regard, PAI-1 binds to and is protected from inactivation by vitronectin, an important component of the extracellular matrix[15]. The cellular nature of the venous occlusions suggests that such a perturbation may have affected the cellular adhesive properties of the venous endothelium.

Regardless of the precise mechanism, however, the results presented here provide strong evidence that elevated PAI-1 is closely associated with the development of venous occlusions. This finding has important implications in the development of approaches to predict, treat, and possibly prevent thrombotic disorders by monitoring and regulating the endogenous fibrinolytic system. □

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Drosophila Krüppel protein is a transcriptional repressor

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KRÜPPEL (*Kr*), one of the zygotically active *Drosophila* segmentation genes, is expressed in a restricted domain during the blastoderm stage of embryogenesis and is involved in the control of development of the thoracic and abdominal segments of the fly[1]. *Kr* encodes a polypeptide containing DNA-binding zinc-finger motifs[2], disruptions of which yield *Kr* mutants[3,4]. We have assayed the transcriptional activities of wild-type Kr protein as well as Lac repressor/Kr fusion proteins in HeLa and CV-1 cells. Wild-type Kr and a Lac–Kr chimaeric protein repressed transcription from reporter promoters in which a consensus Kr binding site derived from sequences within the *even-skipped* promoter[5] had been inserted in an upstream position. We mapped the repression function of Kr to an alanine-rich amino-terminal region of the protein, as a Lac/Kr fusion protein containing only amino acids 26–110 of Kr repressed transcription from a reporter promoter containing

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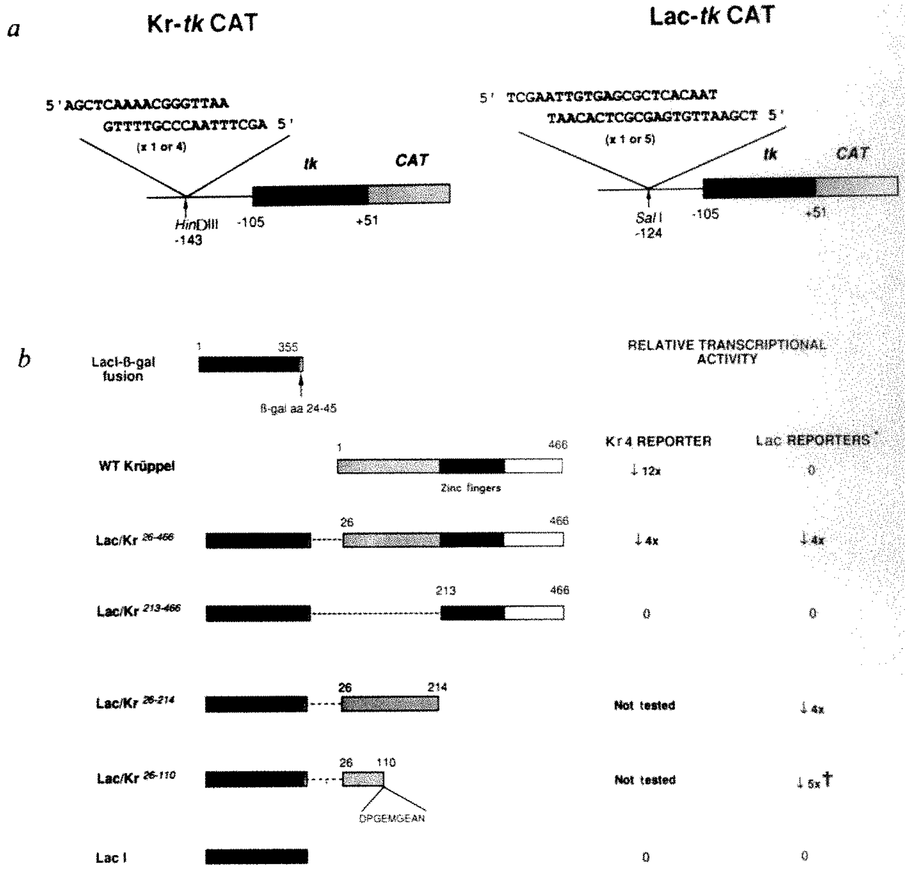

FIG. 1 *a*, Reporter constructs containing either synthetic duplex oligonucleotide Krüppel binding sites or *lac* operator sequences upstream of the HSV *tk* promoter. *b*, The structure of wild-type (WT) Kr and Lac-Kr chimaeric proteins. The table on the right of the figure indicates the relative transcriptional activities of Kr and Lac/Kr chimaeric proteins on the Lac-tkCAT and Kr4-tkCAT reporters as determined from 2-4 independent experiments. *Lac5-tkCAT was used in the demonstration of repression by Lac/Kr$^{26-110}$, whereas Lac1-tkCAT was the reporter gene in all other experiments. No significant difference in fold repression was noted when Lac/Kr$^{26-214}$ was co-expressed with Lac5-tkCAT versus Lac1-tkCAT. †Activities were assayed from transfection into HeLa cells, except for experiments with Lac/Kr$^{26-110}$, which were performed in CV-1 cells.

METHODS. Kr1-tkCAT and Kr4-tkCAT were constructed by the insertion of one or four duplex oligonucleotide Kr-binding sites[5], respectively, into the unique *Hin*dIII site of pBLCAT2 (ref. 21), which contains HSV *tk* sequences from −105 to +51. Lac1-tkCAT and Lac5-tkCAT were constructed by inserting one or five copies of a symmetrical *lac* operator sequence[22] with high affinity for lacI into the *Sal*I site of pBLCAT2. The pBLCAT2 plasmid also contains a wild-type *lac* operator located downstream of the *CAT* gene, within the pUC18 backbone, with an eightfold lower affinity for lacI. The lac repressor was expressed from plasmid pCMVlacI (ref. 22), containing the simian cytomegalovirus promoter. Wild-type *Kr* was expressed from a construct in which *lacI* sequences in pCMVlacI were replaced with the 2.3-kilobase (kb) *Eco*RI insert of pCK2B (ref. 2). The *Lac/Kr* fusion genes were constructed by linking a 1.4-kb *Bgl*II-*Pvu*I fragment encoding lac repressor amino acids 1-355 linked to 23 N-terminal amino acids (aa) of β-galactosidase[23] to portions of the *Kr* cDNA using synthetic duplex oligonucleotide adaptors to maintain the proper reading frame. The adaptors insert two additional amino acids (leucine and glutamic acid) between *lac* and *Kr* sequences. These fusions were cloned into Bluescript SK+ (Stratagene), excised by digestion with *Sac*I and *Eco*RI, then cloned into pCMVlacI digested with *Sac*I and *Eco*RI. The Lac/Kr$^{26-214}$ construct was made by restriction of Lac/Kr$^{26-466}$ at a unique *Bam*HI site at nucleotide 769 of the *Kr* cDNA, followed by end-repair with Klenow fragment and ligation to a linker containing stop codons in all three reading frames[24]. Lac/Kr$^{26-110}$ was constructed by *Bal*31 digestion of Lac/Kr$^{26-466}$, followed by ligation to HSV-*tk* polyadenylation sequences[25]. Nine amino acids (DPGEMGEAN; single-letter code) were added to the Kr amino-acid sequence by its fusion to the HSV-*tk* sequence through a *Bam*HI linker added to the *Sma*I site at nucleotide 1,417 of *tk*, preceding the termination codon. Constructs were confirmed by digestion of plasmids with restriction enzymes and by dideoxynucleotide sequencing of plasmids.

upstream *lac* operators. This demonstrates that the DNA-binding and repression activities of the Kr protein are distinct. These data are consistent with genetic evidence that Kr represses *even-skipped*[6,7] and *hunchback*[8] expression, and suggest that Kr is a negative regulator of transcription in *Drosophila*.

Mutations in *Kr* affect the expression of other gap genes[8,9] as well as pair-rule and homeotic genes[6,7,10,11]. The *Kr* gene product has been identified as a putative transcription factor by virtue of its zinc-finger motifs[2], nuclear location[12], and ability to bind specifically to DNA[5,9,13]. To test for transcriptional activity of the *Kr* gene product, the *Kr* complementary DNA was expressed in human and monkey cells from an expression plasmid containing the simian cytomegalovirus (CMV) promoter. This plasmid was co-transfected with the herpes simplex virus thymidine kinase (HSV-*tk*) promoter, containing one or four *Kr*-binding sites at nucleotide −143 (Kr1-tkCAT and Kr4-tkCAT, respectively) linked to a reporter gene (chloramphenicol acetyltransferase, CAT) (Fig. 1*a*). Expression of *Kr* protein led to a fourfold decrease in CAT activity from Kr1-tkCAT, and a 17-fold decrease in CAT activity from Kr4-tkCAT (Fig. 2*a*), compared with controls of the same reporter constructs co-transfected with a plasmid expressing the lac repressor from the CMV promoter. Cotransfection of the *Kr* cDNA with a reporter construct lacking Kr binding sites did not significantly affect CAT expression (Fig. 2*a*). To localize the region(s) in the Kr protein responsible for repressing transcription from the CAT reporter genes, fusions between sequences encoding portions of the Kr protein and of a heterologous DNA-binding protein, the lac repressor (lacI), were constructed (Fig. 1*b*). A fusion between lacI and amino-acid residues 26-466 of Kr (Lac/Kr$^{26-466}$) also repressed CAT expression from Kr4-tkCAT, whereas a fusion between lacI and residues 213-466 (Lac/Kr$^{213-466}$) of Kr did not (Fig. 2*b*). In addition, Lac/Kr$^{26-466}$ repressed CAT expression from a reporter construct containing an upstream *lac* operator at nucleotide −124 (Lac1-tkCAT), whereas a fusion lacking the N-terminal portion of Kr did not (Fig. 2*c*). The sequence encoding the N-terminal portion of Kr alone, deleting the zinc-finger domain required for DNA-binding activity (see below), was fused to *lacI* by insertion of a translation termination linker after residue 214 of Kr (Lac/Kr$^{26-214}$). Lac/Kr$^{26-214}$, like Lac/Kr$^{26-466}$, repressed transcription from Lac1-tkCAT (Fig. 2*c*). This demonstrates that the DNA-binding and repression functions of Kr are distinct. To further localize the region of Kr responsible for repression, Lac/Kr$^{26-214}$ and Lac/Kr$^{26-110}$ were each transfected along with a reporter construct containing five *lac* operators (Lac5-tkCAT) (Fig. 2*d*). The two fusion proteins repressed CAT expression to similar levels, indicating that an 85-amino acid region of Kr encodes the transcriptional repression activity. None of the Lac/Kr chimaeric constructs repressed CAT expression from a reporter devoid of Kr binding sites or high-affinity upstream *lac* operators (data not shown). Results from all transfections are summarized in Fig. 1*b*.

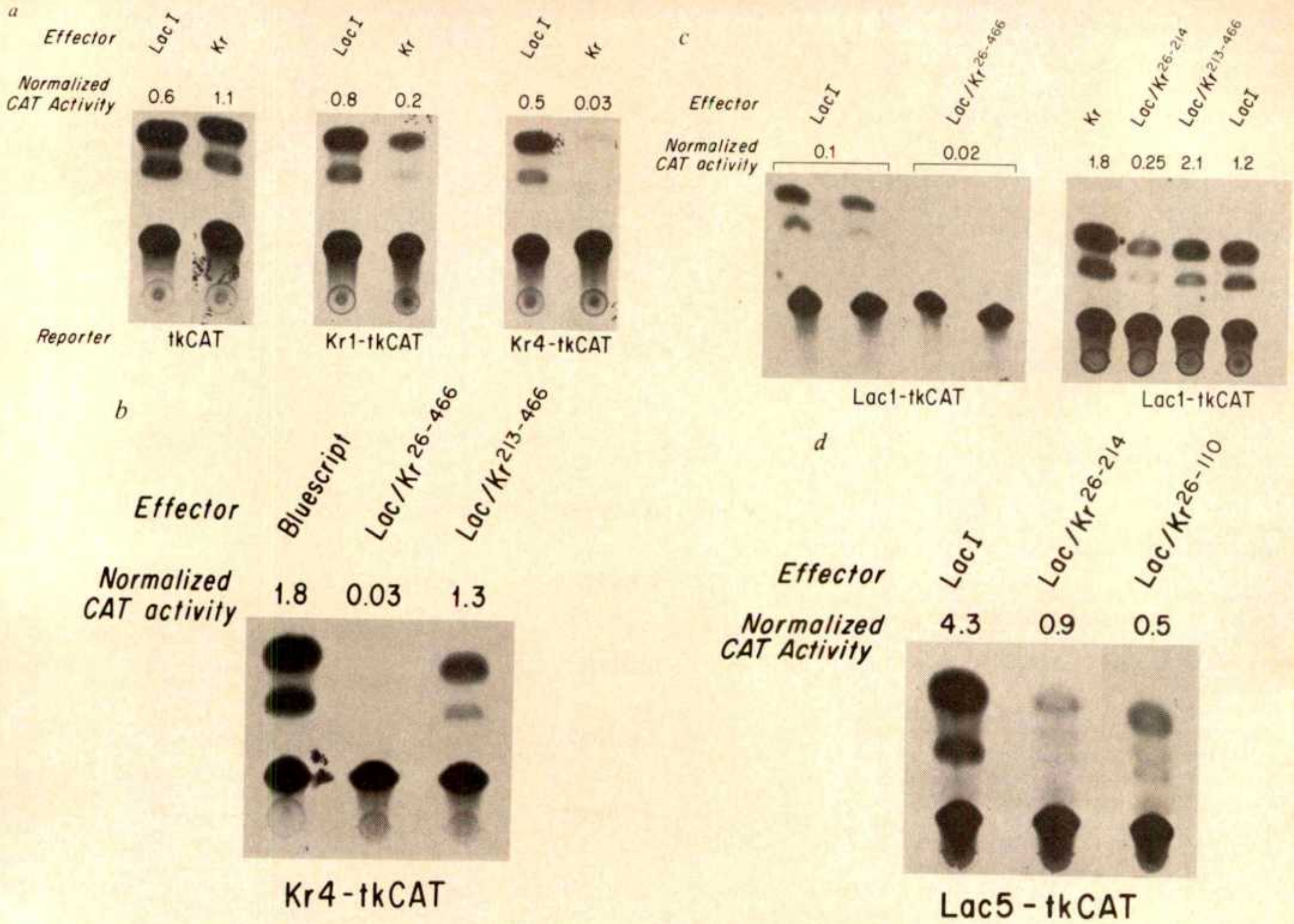

FIG. 2 Repression of CAT expression by Kr and lac repressor–Kr chimaeric proteins. *a*, Effect of wild-type *Kr* or *lacI* effectors on reporter constructs containing zero, one, or four Kr-binding sites upstream of the HSV-*tk* promoter in HeLa cells. *b*, Effect of Lac/Kr chimaeric proteins on the Kr4-tkCAT reporter in HeLa cells. *c*, Effect of Lac/Kr chimaeric proteins on the Lac1-tkCAT reporter in HeLa cells. *d*, Effect of Lac/Kr (N-terminal) chimaeric proteins on the Lac5-tkCAT reporter in CV-1 cells.

METHODS. HeLa cells, adapted to growth in DME medium supplemented with 10% calf serum, were plated at a density of 10^6 cells per 100-mm dish 24 h before transfection. Reporter constructs (5 μg), effector constructs (5 μg) and either 0.5 μg pXHGH5 or 2 μg pTKGH (ref. 26) as an internal control were co-transfected by the calcium phosphate-precipitation method[27]. CV-1 cells were grown in 10% newborn calf serum, plated at a density of 10^6 cells per 100 mm dish and transfected with 7.5 μg of both reporter and effector plasmid constructs, as well as 0.5 μg of pXHGH5. At 45 h post-transfection, cell-growth media were assayed for growth hormone levels[26] and cell extracts were prepared and assayed for CAT activity[27]. The percentage of acetylated chloramphenicol was divided by the growth hormone levels in the media (ng per ml) to normalize CAT activity for the efficiency of transfection. The experiment on the left of *c* was performed in duplicate with results averaged. In addition, the preparation of extracts and performance of CAT assays differed in the two experiments displayed in *c*, accounting for lower levels of CAT activity on the left portion of the figure.

```
a

Kr                 47  AAAIY--PAMGLQQAAAASAFGMLSPTQLLAANRQAAAFMAQLP-MSTLANTLFPHNPAA  103
                       **|||  ***|   **** * |            | ****  *    *   ** *    ****
en                 55  AAGVFHHPAMAFDAAAAAAAAAA 77  229 QQQAAAAAATAAMMLERANFLNCFNPAA  256
(D. melanogaster)                                      | ****  *    *   ** *    ****
en                                                265 QQQAAAAAATAAMMLERANLLNCFNPAA  292
(D. virilis)

b

Kr                      53  AMGLQQAAAASAFGMLSPTQLLAANRQAAAFMA   85
                            *  | **** *  **  *     **   ***  *
eve                    146  AASILQAAANSV-GMPYPPYAPAAAAAAAAAAA  177
```

FIG. 3 Comparison of the primary sequences of the *Drosophila* transcriptional repressors *Kr*, *engrailed* (*en*) and *even-skipped* (*eve*). *a*, Predicted amino-acid sequences from *Kr* (26–110) and *en* were optimally aligned by a computer algorithm that identifies maximally similar subsegments[28]. Identical matches are denoted by (*) and aligned residues that share similar physiochemical properties by (|). This region of Kr could be aligned to two segments of *D. melanogaster en* protein. Only the more C-terminal segment is common to Kr and the *en* protein of both *D. melanogaster* and *D. virilis*. *b*, Alignment found between Kr and *eve*, which overlaps the alignments with *en*. Secondary-structure predictions[29] indicate that these optimally aligned regions are all likely to contain α-helical structures.

The ability of the Lac/Kr fusion polypeptides to bind DNA specifically is necessary but not sufficient to affect repression. Extracts from cells transfected with the Lac/Kr fusion genes supported the formation of specific complexes with *lac* operator DNA (gel-mobility shift assays; data not shown), but only those constructs that contained N-terminal sequences of Kr (for example, Lac/Kr$^{26-214}$ and Lac/Kr$^{26-466}$) led to repression of transcription from reporter plasmids containing *lac* operators (Fig. 1*b*). Similarly, extracts from cells transfected with both Lac/Kr$^{26-466}$ and Lac/Kr$^{213-466}$ formed complexes with Kr-binding-site DNA (data not shown), but only the protein that contained N-terminal sequences led to transcriptional repression

(Fig. 1*b*). This emphasizes the separate nature of the DNA-binding and repression activities of Kr.

The ability of Kr to repress transcription of the reporter constructs described above is consistent with the role of Kr in limiting the boundaries of expression of the gap gene *hunchback* (*hb*)[8] and the pair-rule gene *even-skipped* (*eve*)[6,7] during the blastoderm stage of *Drosophila* development. Our data demonstrate that the Kr protein functions across species lines and contains a transcriptional repression activity, distinct from its DNA-binding activity, that can act at a distance from the start site of transcription. Repression is unlikely to result from steric hindrance, as the Kr and lacI binding sites were placed relatively far from the upstream activator-binding sites of the HSV-*tk* promoter and the start site of HSV-*tk* transcription[14]. Kr repression of the *eve* promoter might occur by competitive binding between Kr and the *hb* protein (Hb) to adjacent binding sites in the *eve* promoter, leading to the displacement of Hb, an activator of the *eve* promoter. Kr bound immediately adjacent to Hb might also mask or quench Hb activity by a protein–protein interaction[5]. Our data are more consistent with a direct model for repression at a distance[15], in which the N-terminal region of Kr would interact with more proximal components of the transcription machinery, such as upstream activator proteins (for example, CTF, TFIID), RNA polymerase II or its accessory proteins, thereby blocking the usual stimulation of transcription.

Comparison of the N-terminal region of Kr between amino acids 26 and 110 with the amino-acid sequence of two other *D. melanogaster* transcriptional repressors, *engrailed*[16,17] and *even-skipped*[17,18], reveals the existence of similar alanine-rich subsegments (Fig. 3). In addition, one region within the *engrailed* protein that is similar to Kr is evolutionarily conserved between *D. melanogaster* and *D. virilis*[19]. Although the functional significance of these alignments is not known, we are analysing deletions, point mutations and exchanges of sequences among these proteins to determine whether the aligned regions encode a common transcriptional repression activity. Such regions could mediate protein–protein interactions necessary to sequester components of the transcriptional machinery from interactions required to initiate transcription.

Genetic data indicate that Kr also acts as an activator of expression of the gap gene *knirps*, the promoter of which has been shown to contain Kr-binding sites[9]. This activation may depend on the context of the *knirps* promoter, and could be encoded by a different region of Kr. The *Ultrabithorax* (*Ubx*) protein both activates the *Ubx* promoter and represses the *Antennapedia* promoter; however, the activation function of the protein can be deleted without affecting repression activity[20]. We note that a C-terminal deletion of Kr after amino acid 377 yields a hypomorphic Kr phenotype[4], yet this region is not required for the repression function of Kr in our assay system.

Note added in proof: Deletion of the alanine-rich region of *eve* that can be aligned with *Kr* impairs the ability of *eve* to function as a transcriptional repressor in cultured *Drosophila* cells (K. Han and J. L. Manley, personal communication). ☐

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Rapid evolution in response to high-temperature selection

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TEMPERATURE is an important environmental factor affecting all organisms[1,2], and there is ample evidence from comparative physiology that species[1,3] and even conspecific populations[4,5] can adapt genetically to different temperature regimes. But the effect of these adaptations on fitness and the rapidity of their evolution is unknown, as is the extent to which they depend on pre-existing genetic variation rather than new mutations. We have begun a study of the evolutionary adaptation of *Escherichia coli* to different temperature regimes, taking advantage of the large population sizes and short generation times in experiments on this bacterial species[6–10]. We report significant improvement in temperature-specific fitness of lines maintained at 42 °C for 200 generations (about one month). These changes in fitness are due to selection on *de novo* mutations and show that some biological systems can evolve rapidly in response to changes in environmental factors such as temperature.

A clone of a single cell in the ancestral line maintained at 37 °C for 2,000 generations was used to found six high-temperature (42 °C) and six control (37 °C) lines. All lines (ancestral, high-temperature and control) were propagated in Davis minimal medium[11] supplemented with 25 μg ml^{-1} glucose. Cultures (10 ml) were maintained by daily serial dilution into fresh medium, permitting a 100-fold increment in biomass ($\log_2 100 = 6.64$ generations of binary fission) each day. The total size of a population maintained under this culture regime cycles from $\sim 5\times10^6$ to $\sim 5\times10^8$ cells. The founding lines possessed one of two arabinose-utilization marker states[9,10], which were used for clonal identification in fitness assays and for monitoring possible cross-contamination between lines. Selective neutrality of these markers at both 37 °C and 42 °C was verified by preliminary experiments and no cross-contamination of lines was observed. Additional phenotypic markers were also used to exclude the possibility of contamination from external sources[9,10]. The use of an ancestral line that was already well-adapted to the culture medium increased the likelihood that temperature-specific adaptations could be detected, as opposed to other beneficial but non-temperature-specific mutations that might enhance adaptation to the culture medium. Also, by studying spontaneous mutants that have increased in frequency gradually by selection rather than mutants obtained by mutagenesis and subsequent screening for extreme phenotypes, one avoids mutations that have profoundly deleterious pleiotropic effects and consequently would have little evolutionary significance.

TABLE 1 Improvements in fitness during 400 generations at 42 °C

Generation	Estimated mean fitness over all lines*	Estimated variance in fitness among lines†
100	$\bar{X} = 0.991$ $t_s = -1.386$ $0.5 < P < 0.9$	$s_g^2 = 2.07 \times 10^{-6}$ $F_s = 1.008$ $0.25 < P < 0.5$
200	$\bar{X} = 1.085$ $t_s = 2.712$ $0.01 < P < 0.025$	$s_g^2 = 4.88 \times 10^{-3}$ $F_s = 6.122$ $P < 0.001$
400	$\bar{X} = 1.072$ $t_s = 2.827$ $0.01 < P < 0.025$	$s_g^2 = 2.92 \times 10^{-3}$ $F_s = 4.082$ $0.001 < P < 0.01$

* The mean and standard error of the mean were calculated from the means of the six fitness assays performed for each of the six lines. The corresponding t-statistic is shown, as is the significance level associated with the one-tailed hypothesis that the mean fitness across all lines relative to the ancestral clone was greater than one. The number of degrees of freedom employed in this test was five, corresponding to one less than the number of lines.

† Variance component due to differences in fitness among lines[14]. The corresponding F-statistic is shown, along with the significance level associated with the hypothesis that this variance was greater than zero. The corresponding degrees of freedom for the numerator and denominator in the F-ratio were 5 and 30, respectively.

After 100, 200 and 400 generations of binary fission (15, 30 and 60 days respectively), single colony isolates from each high-temperature line were made to compete against the ancestral clone possessing the alternative arabinose marker state. These competition experiments used the same culture conditions in which the high-temperature lines had been maintained. Six replicate competition experiments were run for each of the six lines at each sample time. Both competitors were preincubated at 42 °C for one day, such that each had equal opportunity to acclimatize physiologically to the culture conditions. Thereafter equal aliquots of each competitor were added to fresh medium; initial densities were determined by plating on medium that permitted the competitors to be distinguished on the basis of the arabinose-utilization marker state. The mixed culture was maintained at 42 °C for one day and the final densities of ancestral and high-temperature clones determined again by plating. These competition experiments enabled us to examine adaptive evolution (that is, improvement in fitness relative to the ancestral clone) by the high-temperature lines. Fitness (W) was defined as the ratio of the number of doublings for the high-temperature and ancestral clones: $W = \log_2 (N'_f/N'_i)/\log_2 (N_f/N_i)$ where N_i and N'_i are the initial densities and N_f and N'_f are the final densities of the ancestral and high-temperature clones, respectively[9,10]. A value of 1 indicates that two clones have equal fitness; a value greater than 1 indicates that the high-temperature line has adapted evolutionarily.

The major results are given in Table 1. At 100 generations of culture at 42 °C there was no significant increase in fitness nor significant heterogeneity in fitness among the six lines. At 200 generations, and again at 400 generations, fitness had increased significantly relative to the ancestral clone by an average of ~7–8%. Furthermore, analyses of variance indicated significant heterogeneity in fitness among the six lines at 200 generations and again at 400 generations. Thus, new mutations that increased fitness at 42 °C had occurred and reached high frequency within only 200 generations (30 days) in at least some of the high-temperature lines.

The control lines provide evidence that the adaptive evolution observed in the high-temperature lines was temperature-specific (Table 2). The six control lines maintained at 37 °C for 400 generations did not show any significant fitness improvement when competed at 37 °C against the same ancestral clone. Relative to their common ancestor, the fitness at 37 °C of the control lines was significantly less than the fitness at 42 °C of the high-temperature lines (one-tailed t-test; $t_s = 2.515$, 10 d.f., $0.01 < P < 0.025$).

We also determined whether the adaptive evolution observed in the high-temperature lines produced any correlated response in fitness at 37 °C, the ancestral temperature regime (Table 2). A significant fitness advantage was observed when the high-temperature lines were competed against the ancestor at 37 °C. Although the fitness advantage observed at 37 °C (~4%) was less than the advantage observed at 42 °C (~7%), this difference was not statistically significant (one-tailed t-test for paired comparisons, $t_s = 0.898$, 5 d.f., $0.1 < p < 0.25$). Also, mean fitnesses at 37 °C and 42 °C were not positively correlated across the high-temperature lines (coefficient of correlation $r = -0.088$, 4 d.f., $P > 0.5$). One possible explanation for this lack of correlation across lines is heterogeneity among alleles in their effects on a correlated character[9,12]. To examine this possibility we first computed the overall ratio of the correlated response to selection (mean change in fitness at 37 °C, Table 2) to the direct response to selection (mean change in fitness at 42 °C, Table 1): $(1.040 - 1)/(1.072 - 1) = 0.554$. This ratio provides an estimate of the regression of fitness at 37 °C on fitness at 42 °C (see equation 19.5a in ref. 13). We then used the jackknife procedure[14] to determine the influence of each fitness assay on this ratio. An analysis of variance using the jackknifed data (Table 3) indicates significant heterogeneity among the lines in this ratio, and hence in the relationship between fitnesses at 37 °C and 42 °C. Five of the six lines give a positive regression coefficient (mean slope from jackknifed data = 0.782) and among these five lines there is a significant positive correlation between mean fitnesses at 37 °C and 42 °C ($r = 0.968$, 3 d.f., $P < 0.01$). The other line gives a negative regression coefficient (mean slope from jackknifed data = −0.738) that obscures the overall correlation. There is evidently heterogeneity among alleles that improve fitness at 42 °C in their effect on fitness at 37 °C.

Even with such heterogeneity, the average correlated response of fitness at 37 °C for the high-temperature lines was significantly greater than the average direct response of the control lines at 37 °C (two-tailed t-test, $t_s = 2.982$, 10 d.f., $0.01 < P < 0.025$). This unexpected result demonstrates that adaptation to an environment may sometimes occur more rapidly as a consequence of selection in another environment than as a direct evolutionary response. We see three possible explanations for this phenomenon in our experimental system. First, certain mutations may occur only at higher temperatures. Second, the high-temperature regime may have increased the rate of certain mutations[6,15], including some beneficial mutations sufficiently rare that they were not yet observed at 37 °C. Third, equivalent mutations may have occurred at the same rate at 37 °C, but were not fixed during this period because of a smaller selective

TABLE 2 Temperature-specificity of improvements in fitness

Estimated mean fitness over all lines*	Estimated variance in fitness among lines†
Direct response of fitness at 37 °C after propagation at 37 °C for 400 generations	
$\bar{X} = 1.006$ $t_s = 1.164$ $0.1 < P < 0.25$	$s_g^2 = -1.28 \times 10^{-4}$ $F_s = 0.587$ $0.5 < P < 0.75$
Correlated response of fitness at 37 °C after propagation at 42 °C for 400 generations	
$\bar{X} = 1.040$ $t_s = 4.056$ $0.001 < P < 0.01$	$s_g^2 = 2.13 \times 10^{-4}$ $F_s = 1.585$ $0.1 < P < 0.25$

* See Table 1.

† See Table 1.

TABLE 3 Heterogeneity in ratio of correlated response (change in fitness at 37 °C) to direct response (change in fitness at 42 °C) among lines propagated at 42 °C for 400 generations*

Source	Degrees of freedom	Sums of squares	Mean square	F_s
Among lines	5	24.24	4.85	2.76
Within lines	66	115.91	1.76	$0.025 < P < 0.05$
Total	71	140.14		

* The overall ratio of the correlated response (Table 2) to the direct response (Table 1) is calculated in the text. The jackknife procedure[14] was used to compute pseudovalues that indicate the influence of each of the 72 fitness assays (six assays at each of two temperatures for each of six lines) on this overall ratio. The grand mean of the pseudovalues was 0.529, close to the overall ratio. The analysis of variance tests for heterogeneity among lines in pseudovalues. Significant heterogeneity indicates genetic variation among lines in the effects of alleles that improve fitness at 42 °C on fitness at 37 °C. See text for details.

advantage at this temperature (smaller selective advantage both increases the probability that a favourable mutation will be lost initially owing to genetic drift and increases the amount of time that would be required for the favoured mutant allele, if it is not lost by drift, to reach some specified high frequency[16,17]). These results also bear on the issue of whether there necessarily exists a negative genetic correlation (that is, pleiotropic trade-off) between fitnesses measured in different environments (for example, temperatures[18]). The correlated improvement in fitness at 37 °C in most of the high-temperature lines clearly indicates no necessary loss of fitness in the ancestral environment (37 °C) during adaptation to a new environment (42 °C).

Selection studies, in which one monitors the response of laboratory populations to variation in natural environmental variables, may provide a powerful tool to investigate and predict the response of natural populations to environmental variation[19] (for example, climate change[20,21]). Our experiments demonstrate that some bacteria can respond to changes in temperature in ways that improve evolutionary fitness over short absolute time periods. Mutations favouring such fitness improvement are evidently not rare and can spread rapidly through a population. Some organisms may therefore be able to track and evolve in response to temperature changes in their environments even seasonally and certainly over longer time periods. ☐

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Proximity to the promoter inhibits recognition of cauliflower mosaic virus polyadenylation signal

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THE replication of retroviruses and pararetroviruses (such as caulimo- and hepadnaviruses)[1] involves the production of terminally redundant genomic-length RNA (Fig. 1). The sequence repeated at both ends of the RNA (the R region) contains a polyadenylation signal, and for production of full-length RNA the version of this at the 5′ end of the template must be bypassed by RNA polymerase, but the version at the 3′ end must be recognized[2–5]. This implies that the position of the polyadenylation signal determines its efficiency, and we report here experiments aimed at investigating the basis of this phenomenon. Our results with cauliflower mosaic virus[6] suggest that proximity to the transcription initiation site inhibits messenger RNA 3′-end processing directed by polyadenylation signals.

To test whether the cauliflower mosaic virus (CaMV) R region without additional upstream CaMV sequences was sufficient to direct 3′-end processing in plants, we inserted the chloramphenicol acetyltransferase (CAT) reporter gene at the beginning of the R region (plasmid R-CAT, Fig. 2*a*). A DNA fragment from the nopaline synthetase gene (*nos*), containing a second polyadenylation signal[7,8], was placed downstream of R to rescue RNA molecules that were not processed at the CaMV site. Total RNA was purified from transiently transfected plant protoplasts (*Nicotiana plumbaginifolia*) and analysed in RNase protection assays using an antisense probe (P1) that covered both polyadenylation signals (Fig. 2*a*, *b*). Only one major band was obtained, which was of the expected size (190 nucleotides) for a transcript processed at the CaMV site. Processing at this site

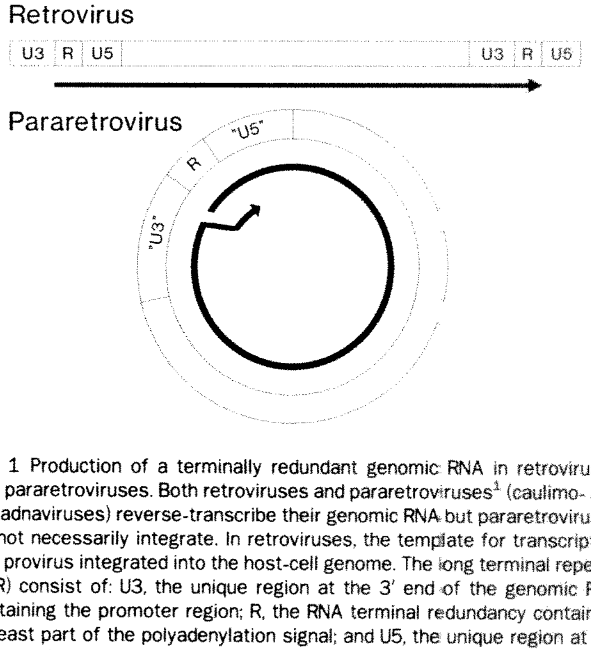
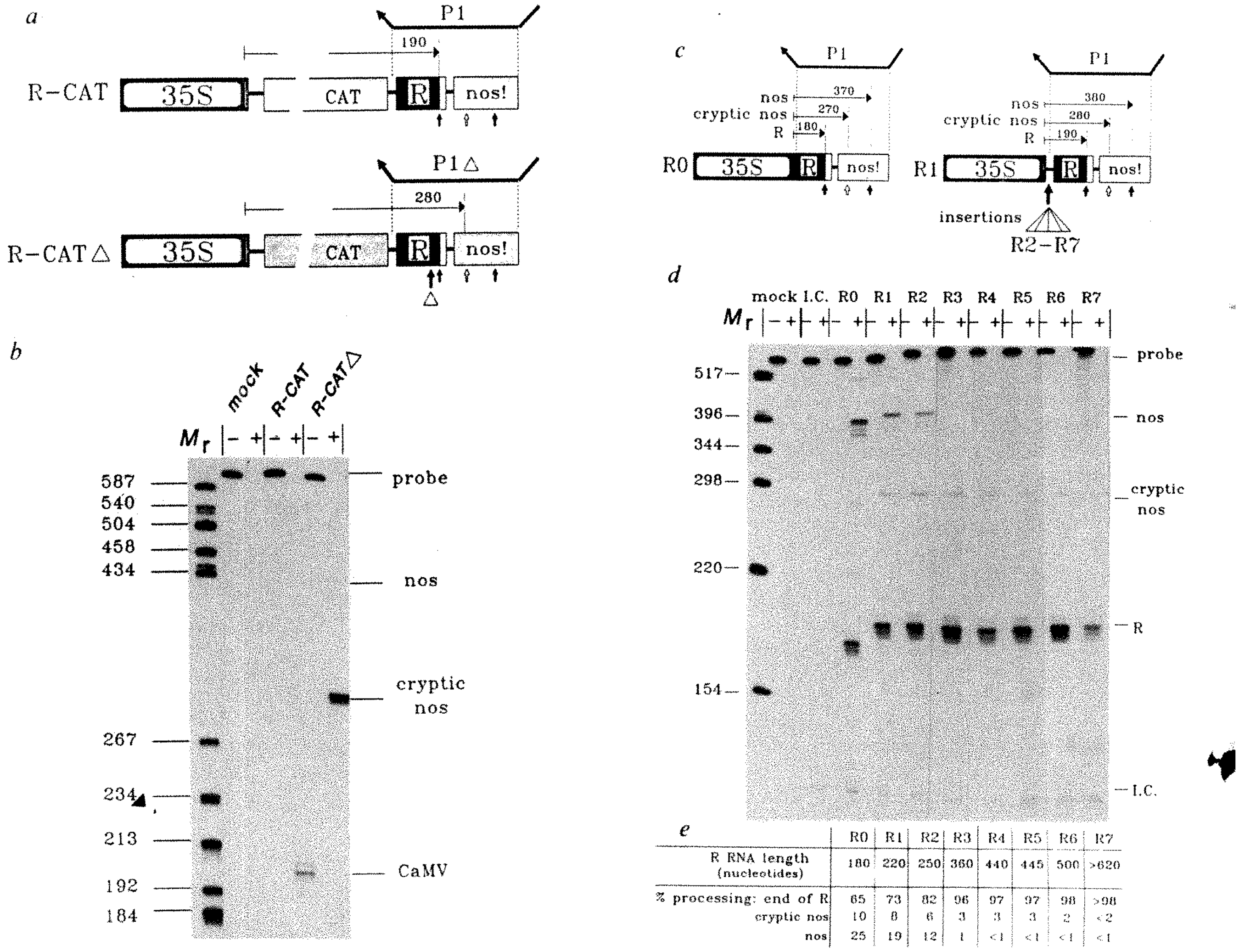


FIG. 1 Production of a terminally redundant genomic RNA in retroviruses and pararetroviruses. Both retroviruses and pararetroviruses[1] (caulimo- and hepadnaviruses) reverse-transcribe their genomic RNA but pararetroviruses do not necessarily integrate. In retroviruses, the template for transcription is a provirus integrated into the host-cell genome. The long terminal repeats (LTR) consist of: U3, the unique region at the 3′ end of the genomic RNA containing the promoter region; R, the RNA terminal redundancy containing at least part of the polyadenylation signal; and U5, the unique region at the 5′ end of the RNA. In cauliflower mosaic virus (CaMV), the template for transcription is a circular mini-chromosome. The genomic 35S RNA and the subgenomic 19S RNA use the same poly(A) addition site[10]. Regions equivalent to the retrovirus U3 and U5 are defined by the start site of (−) and (+) strand DNA synthesis during reverse transcription. In CaMV, 'U3' contains the 35S promoter from which the genomic RNA is transcribed and R contains all the elements necessary for 3′-end processing of the 35S and the 19S RNA.

was abolished on deletion of the consensus hexanucleotide AATAAA (ref. 9) in R (construct R-CATΔ, Fig. 2*b*). A new band of 280 nucleotides was then obtained, the expected size for transcripts reading through R and being processed 20 nucleotides downstream of a cryptic AATAAA in the *nos* signal[7,8]. This AATAAA is located 120 nucleotides upstream of the natural *nos* gene poly(A) addition site. Additional elements within the CaMV R are likely to be responsible for the recognition of this cryptic site in our experiments.

To measure the bypass of the CaMV polyadenylation site at the 5′ end of the genomic RNA, we modified plasmid R-CAT by placing R immediately downstream of the 35S RNA promoter (the CaMV equivalent of U3, Fig. 1). On RNA mapping with probe P1, we detected three bands of the expected size for transcripts processed at the CaMV poly(A) addition site and at the normal and the cryptic sites in the *nos* segment (plasmid R0, Fig. 2*c*, *d*). The proportion of transcripts processed at the CaMV versus the *nos* sites was used as a measure for the bypass of the first signal (Fig. 2*e*). About two-thirds of the transcripts were processed at the end of R and one-third at the two *nos* sites.

To determine whether the inefficient use of the CaMV poly(A) addition site in construct R0 was caused by its proximity to the RNA 5′ end, a 40 nucleotide polylinker was placed at the beginning of R (construct R1). DNA fragments of increasing length, isolated from calf thymus DNA, were inserted into the polylinker creating constructs R2–R7 (Fig. 2*c*). Increasing the



	R0	R1	R2	R3	R4	R5	R6	R7
R RNA length (nucleotides)	180	220	250	360	440	445	500	>620
% processing: end of R	65	73	82	96	97	97	98	>98
cryptic nos	10	8	6	3	3	3	2	<2
nos	25	19	12	1	<1	<1	<1	<1

FIG. 2 Changes in recognition of the CaMV polyadenylation signal on increasing RNA length. *a*, *c*, RNA mapping strategy. The 35S promoter, CAT reporter gene, CaMV R plus 20 nucleotides of CaMV downstream sequences (empty box), and *nos* polyadenylation signal (nos!) are shown. All plasmids except R0 contain a 40-nucleotide polylinker (thick black line) between the third and fourth nucleotide of R, used for insertions. In construct R-CATΔ, the AATAAA deletion is indicated (Δ). Normally used (small black arrows) and cryptic (small open arrow) 3′-end cleavage sites in R and *nos* fragments, transcripts (thin lines and arrows) and 300-nucleotide antisense probes P1 and P1Δ containing part of the polylinker (upper thick line) are shown. Sizes of expected protected fragments are given. *b*, *d*, RNase protection assays. Lane M_r, relative molecular mass markers as ^{32}P-labelled single-stranded DNA fragments. Hybrids are shown before (−) and after (+) digestion. Lane 'mock', transfection in absence of input plasmids. *d*, A patchwork of several exposures of the same gel was assembled to compensate for variation in total RNA recovered. This is measured by comparing the intensity of the band (I.C.) corresponding to a cotransfected internal control plasmid containing a truncated R (without *nos* fragment), partly homologous to probe P1 (120 nucleotides). Lane I.C., transfection with internal control only. *e*, Quantitation of transcripts produced. Percentage of processing: the ratio of the relevant band was determined by optical densitometry (the number of labelled nucleotides in each band was taken into account). Average numbers from this and three other independent experiments are given.

METHODS. DNA constructs: R-CAT, *Eco*RI (35S promoter and CAT gene) and *Eco*RI-*Hin*dIII (R) fragments of pDW2[18] and *Sac*I-*Eco*RI (nosPA) fragment of pBI 101 (ref. 19) into pIC19R$^+$ (ref. 20) polylinker; R-CATΔ, deletion of AATAAA within R by oligonucleotide-directed mutagenesis; probes P1 and P1Δ, *Pst*I-*Eco*RI (R) and *Eco*RI (nosPA) fragments of R-CAT and R-CATΔ into pGEM2 (Promega) polylinker; R0, *Nco*I-*Sca*I fragment (6,909–7,632) of CaMV-S[21] into *Nco*I-*Kpn*I(blunted) fragment of R-CAT; R1, *Eco*RI (35S promoter and R) fragment of pDH51[18] ligated to *Eco*RI fragment of R0, R2–R8, random calf thymus *Sau*3A DNA fragments into *Bam*HI site of R1 polylinker. Protoplast transfection and RNA analysis: *N. plumbaginifolia* protoplasts were transfected and total RNA purified after 6 h and mapped by RNase protection as described[22,23]. DNase treatment was included.

space between the transcription initiation and the CaMV poly(A) addition site resulted in an increased proportion of transcripts processed at the CaMV site (Fig. 2*d* and *e*). A pre-mRNA length of over 600 nucleotides resulted in <2% read-through of the CaMV signal. Sequencing of the inserts revealed no obvious homology to known consensus sequences beneficial for 3′-end processing. It is interesting that increasing the distance between the 35S promoter and the polyadenylation sites also resulted in progressively stronger recognition of the cryptic over the normal *nos* site (Fig. 2*e*). The data shown were obtained with *N. plumbaginifolia* (Solanaceae) protoplasts, which is not a host for CaMV infection. Similar results were obtained with *Orychophragmus violaceus* (Crucifereae) protoplasts, a CaMV host (data not shown).

If these results were to apply to natural CaMV infection of plants, then roughly two-thirds of the initiation events would be aborted by processing and polyadenylation at the 5′ R element, leading to the production of a large amount of 'short stop' RNA. To test this, total RNA was purified from an infected turnip plant and mapped with a probe covering R and 3′ and 5′ flanking sequences (Fig. 3*a*). We expected protected fragments of 180 nucleotides for the short-stop transcript, 220 nucleotides for the 5′ end of the 35S genomic RNA, and 260 nucleotides for the 3′ ends of the 19S and 35S RNAs[10] (Fig. 1). All three fragments were detected in both the total RNA and the poly(A)$^+$ fraction. The 220-nucleotide fragment was, however, also detected in the poly(A)$^-$ fraction, presumably as a result of degradation of the very long 35S RNA (8,200 nucleotides). As the short-stop RNA is present and polyadenylated in an infected plant, it will be interesting to investigate its possible role in CaMV infection.

To determine whether the spacing hypothesis for conditional recognition of a polyadenylation signal is relevant only for the CaMV system or reflects a more general feature of plant polyadenylation signals, the two signal-containing fragments were exchanged. The CaMV signal was then used to rescue the transcripts that bypassed the *nos* signal (Fig. 4*a*). Also, the efficiency of 3′-end processing at the *nos* site depended on its distance from the transcription start site (Fig. 4*b*). The three bands observed confirm the heterogeneity of the normal processing site described earlier[7]. Protected fragments corresponding to transcripts processed at the cryptic *nos* site were not detected in the lower part of the gel (data not shown).

Processing of the nascent RNA 3′ end probably occurs before termination of transcription[11-14]. On this basis, the progressive recognition of a polyadenylation signal with increasing pre-mRNA length could be explained by several mechanisms. One possibility is that the polyadenylation signal of a short transcript is poorly accessible to the processing/polyadenylation complex, perhaps as a result of steric hindrance caused by a capping enzyme bound to the 5′ terminus of the nascent RNA[15-16]; or similarly, the processing/polyadenylation complex might require a long foothold upstream of the poly(A) addition site. Alternatively, one could imagine that the RNA polymerase II initiation complex is converted *en route* to an elongation complex by progressive gain or loss of factors, allowing or preventing recognition of the polyadenylation signal. Denome and Cole[17] proposed that such a factor, either soluble or associated with the RNA polymerase, is involved in polyadenylation. Finally, a minimum mRNA length requirement could provide a simple explanation for the conditional recognition of some other retrovirus or retroelement polyadenylation signals. ☐

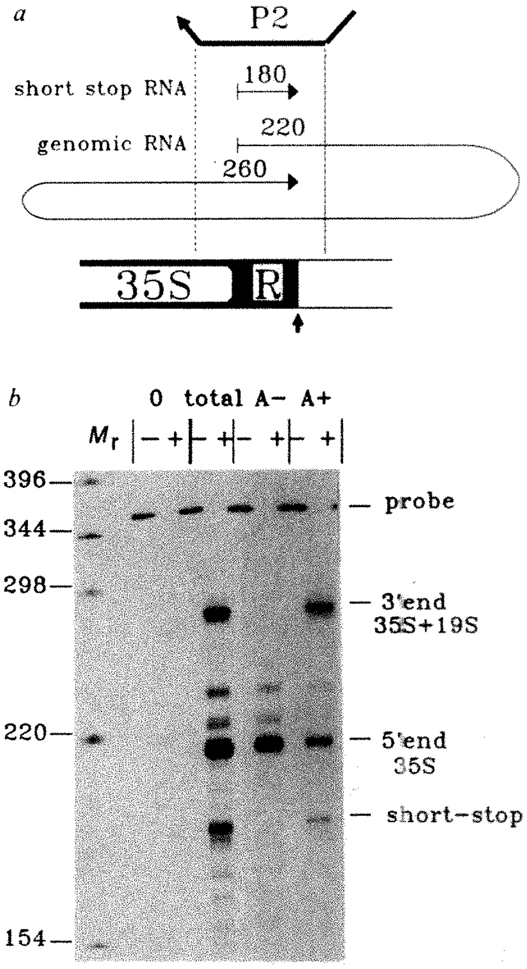
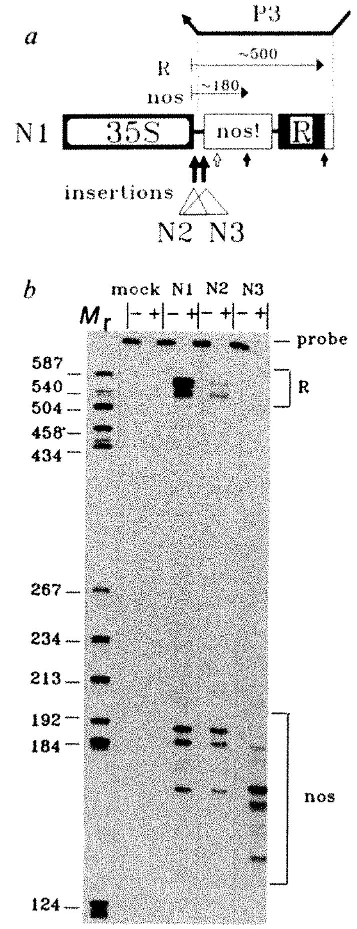


FIG. 3 Detection of the 'short-stop' transcript in an infected plant. *a*, RNA mapping strategy. Relevant CaMV regions (35S promoter and R), the anti-sense P2 probe and 35S genomic and short-stop RNAs with expected sizes of protected fragments are shown (as in Fig. 2). The 3′ end of 19S RNA (not shown) would also give rise to a 260-nucleotide protected fragment. *b*, RNase protection assay as in Fig. 2. Control lane 0, 10 μg transfer RNA was hybridized to probe P2; total, 1 μg total RNA; A$^-$, poly(A)$^-$ fraction from 1 μg total RNA; A$^+$, poly(A)$^+$ fraction from 1 μg total RNA.

METHODS. DNA constructs: Ca100, a CaMV hybrid strain containing the *Bst*EII–*Bam*HI small fragment from CM4184[24] and the small *Bam*HI and large *Bam*HI–*Bst*EII fragments from CaMV-S[18] was used. Probe P2, *Eco*RV–*Bgl*II fragment of Ca100 (part of the 35S promoter, R and 40 nucleotides downstream) cloned into pGEM1 (Promega) polylinker. RNA analysis: total RNA was prepared 2 months after infection of the plant, essentially as described for protoplasts (Fig. 2). Poly(A)$^+$ and poly(A)$^-$ RNAs were fractionated by passage through an oligo(dT) cellulose column (Boehringer). RNA mapping was done as in Fig. 2.



c

	N1	N2	N3
nos RNA length (nucleotides)	200	420	1010
% processing: nos	60	80	>98
end of R	40	20	<2

FIG. 4 Changes in recognition of the *nos* polyadenylation signal on increasing RNA length. *a*, RNA mapping strategy. The elements in each construct are represented as in Fig. 2. Two restriction sites in the polylinker (large black arrows) were used for insertion of sequences to produce N2 and N3. Because the antisense probe P3 (upper thick line) covers most of the polylinker, slightly smaller protected fragments are expected for N3. Sizes of expected protected fragments are indicated above each transcript. *b*, RNase protection assay, as for Fig. 2. *c*, Quantitation of transcripts produced. The *nos* RNA length, distance between transcription start site and normal *nos* processing site. Percentage of *nos* read-through (average of two experiments) was calculated by comparing R transcript intensity with *nos* transcript intensity and accounting for the number of labelled nucleotides in each band.
METHODS. DNA constructs: N1, *Eco*RI-*Pst*I fragment of pDH51[18] (35S promoter), *Pst*I-*Hin*dIII fragment of pNOSCAT[25] (*nos* polyadenylation signal) and *Bam*HI-*Eco*RI fragment of pDH51 (R) cloned into pIC19R$^+$ polylinker[20]; N2, insertion of the calf thymus DNA used in R5 (Fig. 2) into *Bam*HI site of N1 polylinker; N3, exchange of the *Eco*RV-*Pst*I fragment for that of pDW2[18] (containing the CAT gene in *Sal*I site of N1 polylinker); probe P3, *Sma*I-*Eco*RI fragment of N1 into pGEM2 polylinker (Promega). Protoplast transfection and RNA analysis as in Fig. 2.

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Identification of a 32K plasma membrane protein that binds to the myristylated amino-terminal sequence of p60$^{v\text{-}src}$

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THE transforming protein of Rous sarcoma virus, p60$^{v\text{-}src}$, is a myristylated membrane-bound phosphoprotein[1-3]. Interaction of p60$^{v\text{-}src}$ with the plasma membrane is essential for transforming activity[4-5], and is mediated by association with a membrane-bound Src receptor protein[6]. Evidence for the existence of an Src receptor is based on the ability of a myristylated peptide containing the N-terminal Src sequence to inhibit binding of p60$^{v\text{-}src}$ to plasma membranes *in vitro*: binding of p60$^{v\text{-}src}$ to a plasma membrane receptor is therefore mediated by N-terminal Src sequences[6]. Here we report that a myristyl-Src peptide, but not the corresponding non-myristylated peptide, can be specifically crosslinked to a plasma membrane protein of relative molecular mass 32,000 (M_r 32K). The 32K protein represents an Src-binding protein in the plasma membrane that is likely to be a component of the myristyl-Src receptor, and which could be involved in cellular transformation.

We have employed an N-terminal Src peptide to identify the Src receptor by chemical crosslinking according to the strategy outlined in Fig. 1. A myristylated Src peptide (MGYsrc) and the corresponding non-myristylated Src peptide (GYsrc) were synthesized[7], consisting of amino acids 2-12 of the mature Src polypeptide sequence with a C-terminal tyrosine residue appended. Both peptides were iodinated on tyrosine with chloramine T to a specific activity of 5×10^5 c.p.m. μg^{-1}. The ^{125}I-labelled peptides were incubated with plasma membranes from uninfected vole fibroblasts, the bifunctional crosslinking reagent BS^3 (Fig. 1) was added, and membranes were re-isolated by ultracentrifugation. When membrane-bound material was analysed by SDS-PAGE and autoradiography, a prominent iodinated band of M_r 33K was evident in membranes incubated with myristylated, but not with non-myristylated Src peptide (Fig. 2). Radiolabel incorporation into the 33K band was inhibited by non-radioactive MGYsrc peptide, but not by GYsrc peptide (Fig. 2). In addition, myristyl-peptides containing the N-terminal sequences of other known myristylated proteins (cyclic AMP-dependent protein kinase[8] and Gag polyprotein of murine leukaemia virus[9]) did not inhibit radiolabelling of the 33K band by iodinated MGYsrc (results not shown). The 33K band did

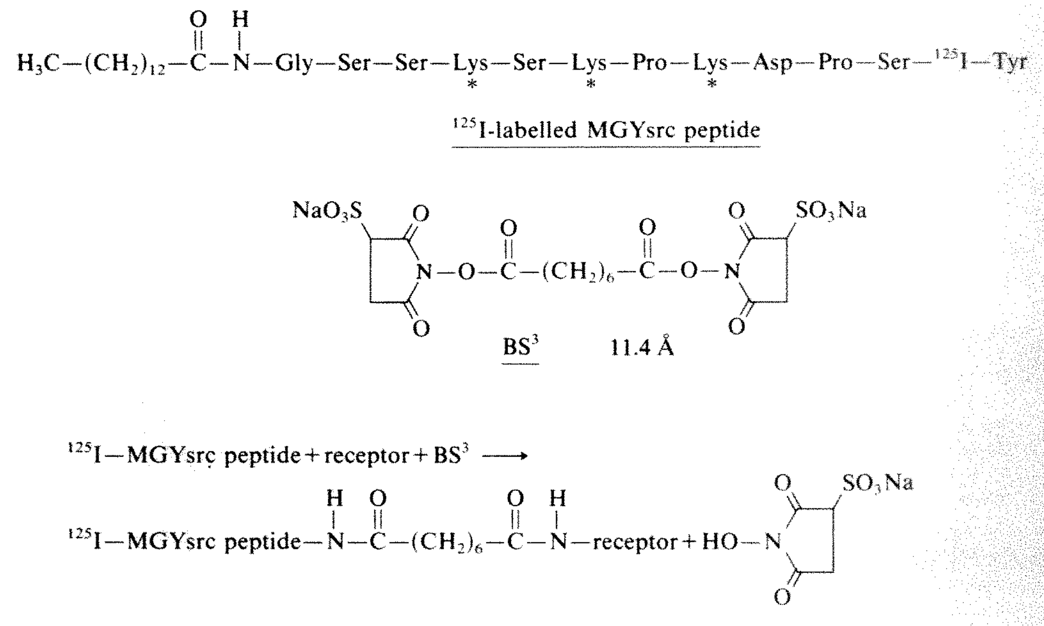
H₃C—(CH₂)₁₂—C(=O)—N(H)—Gly—Ser—Ser—Lys—Ser—Lys—Pro—Lys—Asp—Pro—Ser—¹²⁵I—Tyr
¹²⁵I-labelled MGYsrc peptide
NaO₃S
SO₃Na
BS³
11.4 Å
¹²⁵I—MGYsrc peptide + receptor + BS³ ⟶
¹²⁵I—MGYsrc peptide—N—C—(CH₂)₆—C—N—receptor + HO—N

FIG. 1 Crosslinking strategy. The homobifunctional N-hydroxysuccinimide ester, bis(sulphosuccinimidyl) suberate (BS^3) was used to covalently crosslink iodinated myristyl-Src peptide (MGYsrc) to a membrane receptor by reaction with available amino groups. The MGYsrc peptide contains three potential sites for reactivity (epsilon amino groups of Lys, *).
METHODS. Myristyl (MGYsrc) and non-myristyl (GYsrc) peptides, synthesized as described[7] were iodinated on the C-terminal tyrosine residue with Na^{125}I and immobilized chloramine T (IODO-BEADS, Pierce), and were purified by chromatography through Sephadex G-10.

not represent an aggregate of crosslinked peptide alone, as no label was incorporated into a complex of this M_r in the absence of membranes (Fig. 2). Because the M_r of the MGYsrc peptide is ~1K, the M_r of the Src-binding protein detected in Fig. 2 is 32K.

Formation of a covalent complex between MGYsrc peptide and the 32K protein did not occur in the absence of a chemical crosslinker (Fig. 3*a*, lane 1), but was not limited to the use of BS^3 as a crosslinking agent. A 33K crosslinked product was also produced when a disulphide-containing derivative of BS^3, DTSSP, was employed (Fig. 3*a*, lane 5). As predicted, cleavage of the disulphide bond in DTSSP with free thiol resulted in dissociation of the complex between ^{125}I-labelled MGYsrc and the 32K protein (Fig. 3*a*, lane 4). Moreover, the heterobifunctional crosslinker MBS also resulted in attachment of ^{125}I-labelled MGYsrc to the 32K protein (Fig. 3*a*, lane 3). As MBS specifically crosslinks ligands containing primary amino groups to those containing sulphydryl groups, and the MGYsrc peptide contains no cysteine residues, the 32K membrane protein must contain a cysteine at or near the myristyl-peptide binding site. Finally, a similar 33K complex was formed between ^{125}I-labelled MGYsrc peptide and a 32K protein in membranes from vole fibroblasts transformed with Rous sarcoma virus (clone 1T) and from uninfected 3T3 fibroblasts, but not from membranes of *Escherichia coli* (Fig. 3*b*).

To better define the nature of the Src-binding protein, the 33K complex was analysed qualitatively and quantitatively. Formation of the 33K complex was sensitive to previous treatment of the membranes with trypsin (400 μg ml^{-1} for 30 min at 37 °C; 60% reduction of binding) or heat denaturation (3 min at 90 °C; 95% reduction), confirming that the 32K binding component is a protein. In uninfected vole cells, the distribution of the 33K complex paralleled that of 5′ nucleotidase, a plasma membrane marker enzyme[10] (Fig. 4*a*). This agrees with previous results showing MGYsrc peptide inhibits binding of p60$^{v\text{-}src}$ only in the plasma membrane fraction[6]. The amount of crosslinked MGYsrc peptide in the 33K complex was proportional to the amount of plasma membrane protein in the reaction (Fig. 4*b*). Moreover, addition of increasing amounts of ^{125}I-labelled MGYsrc to a constant amount of membrane protein demonstrated that formation of the 33K complex was saturable, with half-maximal binding occurring at ~6 μM peptide (Fig. 4*c*). These results indicate that binding occurs to a limited number of high-affinity sites, and is consistent with the interpretation that the 32K binding protein is a Src receptor. On the

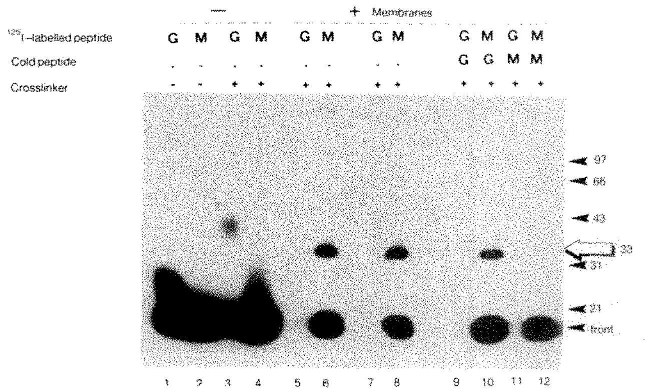

FIG. 2 Crosslinking of ^{125}I-labelled Src peptides to vole fibroblast plasma membranes. Odd-numbered lanes, ^{125}I-labelled GYsrc peptide. Even-numbered lanes, ^{125}I-labelled MGYsrc peptide. Lanes 1, 2, incubation in buffer; lanes 3, 4, incubation in buffer followed by 50 μg ml^{-1} BS^3 crosslinker. Reaction products from lanes 1–4 were run directly on the gel. Lanes 5, 6, incubation with membranes, followed by 50 μg ml^{-1} BS^3 crosslinker; lanes 7, 8, as lanes 5 and 6, respectively, except that the concentration of BS^3 crosslinker was 100 μg ml^{-1}, lanes 9, 10, as lanes 7 and 8, except that 250 μg ml^{-1} non-radioactive GYsrc peptide was added to the starting incubation mixture; lanes 11, 12, as lanes 7 and 8, except that 250 μg ml^{-1} non-radioactive MGYsrc peptide was added to the starting incubation mixture. Only membrane-bound material is depicted in lanes 5–12; no 33K band was evident in the supernatant. The positions of the M_r markers (in K), as well as the dye front and the 33K complex are indicated in the right margin. Free peptide runs at the dye front. Little GYsrc peptide binds to the membranes, and consequently is not evident at the dye front in samples where membranes were re-isolated. A small amount of GYsrc became crosslinked in the absence of membranes (lane 3). This complex did not bind to membranes and migrated with a slower mobility than the 33K MGYsrc complex.

METHODS. Each reaction contained 100 μl HNE buffer (10 mM HEPES (pH 7.4), 100 mM NaCl, 1 mM EDTA) or uninfected vole (*Microtus agrestis*) P100 plasma membranes in HNE (1 mg ml^{-1}), isolated as described[12]. Iodinated peptide (4×10^5 c.p.m. in 2 μl) was incubated with the membranes for 15 min at 20 °C, followed by 5 or 10 μl BS^3 crosslinker (1 mg ml^{-1} freshly prepared in dH$_2$O) for 15 min at 20 °C. Membranes were re-isolated by ultracentrifugation at 100,000 *g* for 15 min at 4 °C in a Beckman TL tabletop ultracentrifuge. The pellet was solubilized by sonication and boiling in SDS-PAGE sample buffer[13], and released material analysed on a 10% acrylamide gel and autoradiography. Exposure was for 14 h at −80 °C with intensifying screen.

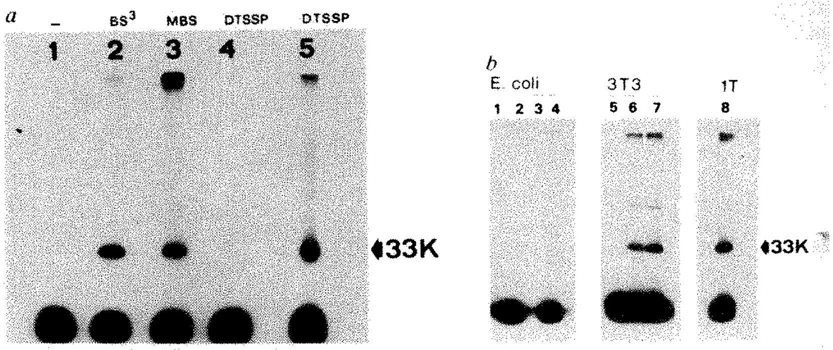

FIG. 3 The 33K complex can be formed with several different crosslinkers in plasma membranes from different species. *a*, Uninfected vole plasma membranes were incubated with ^{125}I-labelled MGYsrc, followed by incubation in the presence or absence of crosslinking reagent. Lane 1, no crosslinker; lane 2, 100 μg ml^{-1} BS^3; lane 3, 100 μg ml^{-1} *m*-maleimidobenzoyl-*N*-hydroxysulphosuccinimide ester (sulfo-MBS); lane 4, 100 μg ml^{-1} 3,3'-dithiobis (sulpho-succinimidyl-propionate) (DTSSP); lane 5, as lane 4 except no reducing agent (β-mercaptoethanol) was added to the electrophoresis sample buffer. Higher M_r oligomers (>300K), which did not enter the separating gel, were sometimes apparent (lanes 3 and 5). Exposure was for 21 h at −80 °C with intensifying screen. *b*, Lanes 1-4, *E. coli* membranes incubated with ^{125}I-labelled GYsrc (lanes 1 and 3) or ^{125}I-labelled MGYsrc (lanes 2 and 4) in the absence (lanes 1 and 2) or presence of 100 μg ml^{-1} BS^3. Lanes 5-7, 3T3 fibroblast plasma membranes incubated with ^{125}I-labelled MGYsrc in the absence (lane 5) or presence of 100 μg ml^{-1} BS^3 (lanes 6 and 7). Lane 8, plasma membranes from Rous sarcoma virus-transformed vole fibroblasts (clone 1T) incubated with ^{125}I-labelled MGYsrc and 100 μg ml^{-1} BS^3.

METHODS. Each reaction contained 100 μl membranes (30–40 μg) prepared from mammalian cells as a P100 fraction[12] or from *E. coli* as a total membrane fraction by osmotic shock[14]. Reaction conditions were as described (Fig. 2).

basis of the data in Fig. 4*c* and the specific activity of the iodinated peptide, we calculate that there are ~10×10^6 molecules of the 32K protein per cell, a number sufficient to accommodate the total number of p60$^{v\text{-}src}$ molecules estimated to be localized to the plasma membrane (1×10^6–2×10^6 per cell[11,12]).

One would predict that in cells transformed with Rous sarcoma virus, the number of available binding sites for ^{125}I-labelled MGYsrc would be lower than in normal cells, as many of the potential binding sites would be occupied by endogenous p60$^{v\text{-}src}$. To test this hypothesis, a titration experiment identical to Fig. 4*c* was performed on plasma membrane fractions from transformed vole cells (clone 1T). Formation of the 33K complex was saturable, and half-maximal binding occurred at 6 μM peptide. But the amount of 33K complex formed at saturation was 23% lower (±7%) than that detected in a parallel experiment with an identical concentration of nontransformed vole cell plasma membranes. Assuming the amount of plasma membrane protein is roughly the same in normal and transformed cells, and that the plasma membrane contains 10×10^6 molecules of the 32K protein, the presence of 2×10^6 molecules of p60$^{v\text{-}src}$ would be expected to reduce MGYsrc peptide binding by 20%, a value that agrees well with the results presented above. As the N-terminal 12 amino acids of the cellular protein p60$^{c\text{-}src}$ are identical to that of p60$^{v\text{-}src}$, the 32 K protein should also function as a receptor for p60$^{c\text{-}src}$. In uninfected cells, the calculated number of 32 K molecules is 100-fold greater than the number of p60$^{c\text{-}src}$ molecules, implying that the 32 K protein may function to bind other proteins in addition to p60$^{c\text{-}src}$.

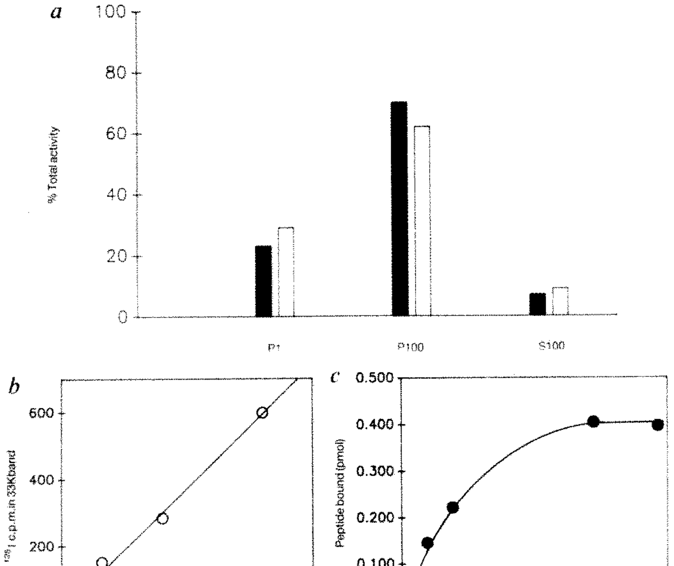

FIG. 4 Distribution and quantitation of the 33K complex. *a*, Crosslinking of ^{125}I-labelled MGYsrc to form the 33K complex (filled bars) and 5' nucleotidase activity (a marker for plasma membrane; open bars) were quantitated in nuclear (P1), plasma membrane-enriched (P100) and cytosolic (S100) fractions of uninfected vole fibroblasts. Each bar represents the average of duplicate experiments; standard deviations were ±5% or less. *b*, *c*, Formation of the 33K complex is dependent on the concentration of plasma membrane protein (*b*) and is saturable (*c*).

METHODS. *a*, Uninfected vole fibroblasts were fractionated in HEPES-containing buffers as described[12], and each fraction was resuspended in an equal volume of HNE. 5' nucleotidase activity was quantitated as described[10]. *b*, Each reaction contained the indicated amounts of uninfected vole fibroblast plasma membrane protein and 4×10^5 c.p.m. ^{125}I-labelled MGYsrc peptide. *c*, Each reaction contained 5 μg uninfected vole fibroblast plasma membrane protein and the indicated concentration of ^{125}I-labelled MGYsrc peptide. Reactions were performed as described (Fig. 2). The 33K band was excised from the dried gel and counted in a gamma counter.

Taken together, these data indicate that the 33K band represents crosslinking of the myristylated Src peptide to a component of the plasma membrane Src receptor. Both fatty-acid and amino-acid sequence moieties of p60$^{v\text{-}src}$ contribute to receptor recognition and binding. Receptor-mediated binding may therefore represent a general mechanism for localizing myristylated proteins to specific cellular compartments. We propose that the myristylated N-terminus of p60$^{v\text{-}src}$ is recognized by and binds to the Src receptor, and thus becomes anchored to the plasma membrane. Proof that the protein identified in this study mediates receptor function awaits isolation, purification and reconstitution of the 32K protein. ☐

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Weak-affinity chromatography

D. Zopf and S. Ohlson

Weak-affinity chromatography is a new method using readily reversible biospecific recognition as the basis for chromatographic separations.

A NOVEL approach to affinity chromatography has emerged in recent years, taking advantage of weak (readily reversible) biospecific recognition as the basis for chromatographic separations[1]. Since its inception some 20 years ago, affinity chromatography has found application mainly as a small-scale, laboratory research operation[2]. The standard scheme is basically an on/off procedure, using high-affinity (association constant, $K_{ass} > 10^5$ M^{-1}) biomolecular recognition between the substance to be purified (the ligate) and the corresponding immobilized interactor (the ligand). The procedure has never gained wide acceptance either as a general analytical tool or in large-scale industrial operations, primarily because of high cost, low throughput, poor recovery of bioactivity, and lack of resolving power.

The stage has been set for a revolution in affinity chromatography by the simultaneous development of macroporous silica/polymer supports for HPLC[3], highly efficient chemistries for coupling ligands, and ready access to multi-milligram amounts of proteins or peptide fragments produced by cellular or molecular cloning techniques. Instead of a process whereby substances are extracted from the mobile phase by virtue of high-affinity interactions with an immobilized ligand, the new approach uses weakly interactive systems where, typically, $K_{ass} = 10^2$–10^4 M^{-1}. Porous matrices comprised of small particles (D_p = 5–10 μm) provide large available surface areas for ligand coupling, permitting ligand loads of, for example, proteins, up to 100 mg ml^{-1} column bed, while maintaining relatively short diffusion distances between active ligands. When purified and concentrated in an immobilized form, weak-affinity ligands can effect rapid and highly specific separation of ligates as retarded peaks under near physiological conditions, readily adaptable to quantitative measurement and experimental control.

Chromatographic perspectives

Weak-affinity chromatography based on biospecific recognition differs from other forms of interactive chromatography such as ion exchange and reversed phase only in the nature of the interaction between analyte and matrix, and is best understood in terms of basic chromatographic theory[4]. The capacity factor (k') that determines retention of a particular ligate is directly related to the amount of active immobilized ligand (L) as well as to the affinity between the ligand and ligate, according to the following equation:

$$k' = C[\mathrm{L}]K_{ass} \quad (1)$$

where C is a constant (with a value usually in the range 0.5–1.0) that reflects column features such as void fraction, porosity and density. Equation 1 is operative as long as the concentration of ligate at injection (c_o) is such that the product $K_{ass}c_o << 1$, that is, the Langmuir adsorption isotherm for the system is within the linear range. It is worth noting that as affinity decreases, retention also decreases, but, at the same time, higher concentrations of injected ligate can be accommodated without loss of performance due to sample overload. Thus, in principle, weak-affinity chromatography favours rapid throughput and process scale-up. From equation 1, it is clear that significant retention (k' = 1–10) in a weak-affinity system depends upon a high concentration of active ligand coupled to the solid phase. For example, to achieve $k' = 1$ with an immobilized IgG monoclonal antibody (M_r = 160 kD) at $K_{ass} = 10^3$ M^{-1} and $C = 1$, the required concentration of antibody on the column bed is approximately 75 mg ml^{-1}.

Weak-affinity separations

An example of chromatography on a 2-ml column loaded with 86 mg of monoclonal IgG coupled to tresyl-activated 10 μm porous silica particles is shown in Fig 1. Results from this and similar experiments confirm theoretical predictions that weak-affinity separations can have good chromatographic efficiency (narrow peaks and high plate numbers). Use of the weak-affinity approach overcomes the poor performance of traditional affinity chromatography, which is mainly due to slow desorption (desorption rate constant: $k_d << 1$ s^{-1}). The chromatographic performance of weak-affinity chromatography actually improves as the strength of ligand/ligate interactions diminishes, in sharp contrast to traditional immunoassays and ligand-receptor binding assays, which are designed to perform with maximal efficiency for measurement of high-affinity ($K_{ass} > 10^6$ M^{-1}) recognition events.

A striking prediction from theory is that weak-affinity chromatography has the potential to resolve completely several similar molecular species present in a crude extract. Figure 2 shows computer-generated elution profiles for small molecular weight ligates with differing weak affinities ($K_{ass} = 10^2$–5×10^3 M^{-1}) for monoclonal IgG immobilized on an HPLC column. For this simulation, a suitable separation 'window' is defined over a range of capacity factors k' = 1–12. The

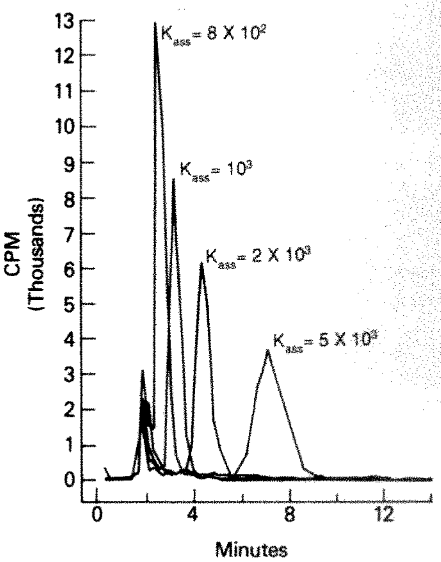

FIG. 1 Weak-affinity chromatography of the tritiated alditol of a glucose-containing tetrasaccharide, $(Glc)_4$, on a 2-ml tresyl-activated SelectiSpher 10 macroporous silica HPLC column coated with IgG[1].

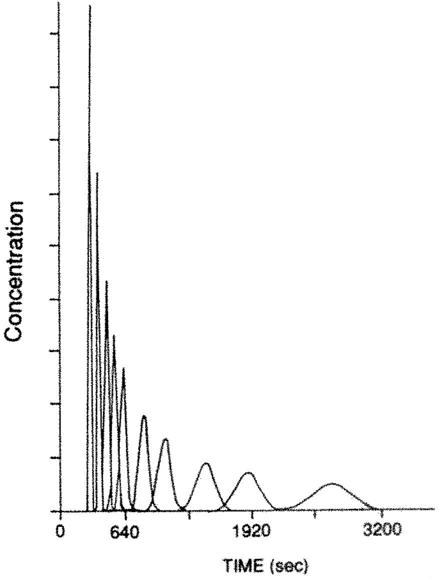

FIG. 2 Computer-generated chromatogram of weak-affinity chromatography of small ligates (M_r = 600 D) on an HPLC column coated with immobilized monoclonal IgG antibody. K_{ass}, varied between 10^2 and 5×10^3 M^{-1}.

first and second peaks represent non-retarded and very weakly interacting species ($K_{ass} < 5 \times 10^2\ M^{-1}$) which, in practice, elute as a single 'void-volume' peak. The subsequent eight peaks illustrate the theoretical potential for weakly bound, 'cross-reacting' ligates to be separated isocratically by more than 90 per cent in less than one hour by HPLC. Figure 3 shows isocratic separation of the α- and β-*p*-nitrophenyl glycosides of maltose dissolved in a crude extract of fetal bovine serum using an HPLC column loaded with a monoclonal IgG antibody that binds the ligates with $K_{ass} = 10^3$–$10^4\ M^{-1}$.

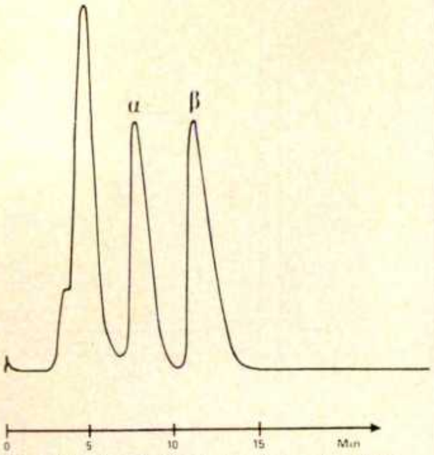

FIG. 3 Weak-affinity chromatography of the α- and β-*p*-nitrophenyl glycosides of maltose. 20 μl of sample containing 0.1 μg of each glycoside dissolved in a crude extract of 4 % fetal bovine serum was injected onto a 10 cm × 5 mm SelectiSpher 10 HPLC column coated with immobilized IgG monoclonal antibody. The column was eluted at 1 ml min^{-1} with 0.02 M sodium phosphate, 0.2 M sodium chloride, pH 7.5 at 45 °C and the eluate monitored for absorption of light at 300 nm.

Is weak binding specific?

The general observation that non-specific adsorption of solutes to chromatography support media is often of weak affinity does not support the converse statement that weak-affinity interactions are, by nature, non-specific. The specificity experienced in weak-affinity chromatography is the cumulative result of many serial weak-binding events whose kinetics, although readily reversible, depend upon binding energies between ligand and ligate that are significantly greater than the random weak interactions between biomolecules. The practical consequences of this specificity are evident in analyses for a specific oligosaccharide performed on crude samples such as human urine or serum[5], where the analyte in such analyses appears as a single retarded peak, well-separated from hundreds of other compounds present at similar or much higher concentrations.

Future trends

As a research tool, weak-affinity chromatography is ideally suited to identification, isolation and systematic investigation of the components of weak bioaffinity reactions. For example, it may offer a means to define the targets recognized by 'lectin-like domains' of cell-surface glycoproteins isolated from membranes of interacting cells.

As a diagnostic tool, weak-affinity chromatography can measure multiple related, or (by combining appropriate ligands) multiple unrelated molecules in complex mixtures within a few minutes. In this respect, it promises to extend the application of immunoassays to families of highly similar molecules that are difficult to discriminate by traditional methods.

As a preparative tool, weak-affinity chromatography enables purification under mild buffer conditions, preserving the bioactivity of molecules. The potential of the weak-affinity method will be more fully realized when macroporous supports are coated with active fragments of macromolecules, such as variable-region, heavy-chain fragments representing weak-affinity monoclonal antibodies. This approach will permit significantly higher active ligand loads, improve preparative capacity and extend the usable affinity range. ☐

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What's new in chromatography

A column designed to eliminate the problem of charge interaction and a preparative/analytical high-performance centrifugal partition chromatography system — highlights of this week's chromatography/HPLC feature.

FOR sample injection and complex liquid handling tasks, E. Merck recommends its LiChroGraph (AS-4000) **autosampler for HPLC** (*Reader Service No. 101*). Offering a 150 standard-vial capacity or 200 micro vials, the LiChroGraph provides three types of sample application: complete or partial filling of the loop, as well as micro-application for virtually zero sample loss, says E. Merck. The autosampler can also be used to perform sample dilution steps before analysis, pipetting and mixing during pre-column derivatization. The addition of internal standards and the take-up and injection of a second phase in two-phase systems are additional features. All instrument functions, from simple needle movements to complex valve switching, are fully programmable. Run programs can be stored in the autosampler's memory. Full PC control of the instrument via a RS232C interface allows the storage of large numbers of application-orientated programs in the PC's memory.

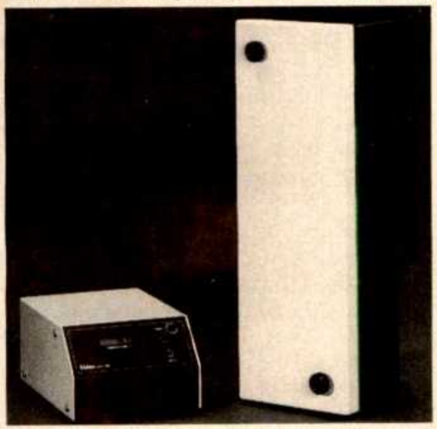
Merck's autosampler with 150-vial capacity.

Severn Analytical's SA 6160 **programmable autosampler** for HPLC has a 160-sample capacity and provides round-the-clock unattended analyses (*Reader Service No. 102*). The autosampler's injection mechanism is designed in such a way as to make the injection needle an integral part of the sample loop; this set-up eliminates carryover and ensures that the sample needle is continually flushed out with mobile phase. Injection volumes from 0.1 to 250 μL can be selected individually for each vial, with a reproducibilty of better than 0.3 per cent standard deviation, says Severn. Using a hand-held control panel, users can program the SA 6160 with run parameters for run time, injection volume, number of injections and timed events. Parameters can also be changed during the run. Practical options include a thermostatted column compartment, column switching valves and a pre-column derivatization facility for amino acid analysis.

Accessories or necessities?

Plasti-Flex **chromatography columns** from Kontes with polycarbonate plastic barrels are designed to eliminate the problem of charge interaction between glass and some biomolecules (*Reader Service No. 103*). Stated applications for the columns include gel filtration, ion exchange, hydrophobic interaction and affinity chromatography. The columns are available in lengths ranging from 5 to 120 cm. Flow adapters can be supplied for columns of 1.0-, 1.5- and 2.5-cm diameter. Plasti-Flex columns are, says Kontes, typically used in aqueous mobile phases at pressures of one bar or less. For easy and secure connection to reservoirs and tubing, the column's polyethylene reservoir cap and bed-support cap have integral polypropylene luer-lock fittings.

For temperature control from 5 °C above ambient to 150 °C, Eldex Laboratories offers a new **chromatography column heater** (*Reader Service No. 104*). The heater can hold up to four 30-cm length columns plus guard columns. To accommodate columns of all sizes, Eldex provides an assortment of interchangeable column inserts. The heater unit is insulated to protect the heater's outer casing and to maximize temperature stability, says Eldex. An injector valve can be installed for the preheating of samples.

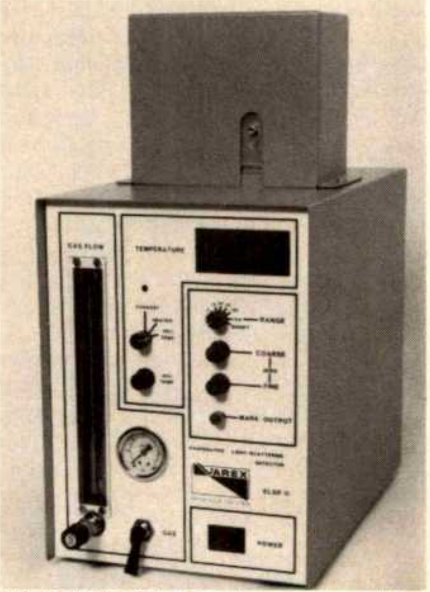

Eldex column heater — heats to 150 °C.

Solvent degassing, an important step for achieving clear chromatographic separations, need not be a problem with Knauer's **on-line degasser for HPLC** (*Reader Service No. 105*). Degassing is particularly important when using RI detectors, which are highly sensitive to undefined gas contents of solvents, says Knauer. Dissolved gases can cause micro bubbles in the pump and detector, which can result in baseline drift. Using Knauer's method, which the company claims is less expensive than helium degassing and more efficient than ultrasonic methods, the solvent to be degassed flows through a gas-permeable Teflon membrane system contained within a vacuum chamber. The pressure differential causes the gas molecules to escape through the Teflon membrane. According to Knauer, the slower the solvent passes through the membrane system, the more efficient the degassing process. Up to three channels with a flow rate of 5 ml min^{-1} each can be used for either one gradient system or three isocratic systems.

Detection systems

Wyatt Technology has launched the new Wyatt/Optilab 903 **interferometric detector** with nanogram sensitivity in most HPLC applications (*Reader Service No. 106*). The 903 can be used for on-line operation by coupling it to any HPLC or GPC/SEC line, or any other light-scattering detector, to create a powerful macromolecular characterization system. Conventional RIs require recalibration after a solvent change is made because of temperature instabilities, but the 903 eliminates the problem by using a circulating water bath as a heat exchanger. Wyatt's 903 detector can also be used for off-line operation as a differential refractometer to determine the specific refractive index increment, *dn/dc*. In this case, it is used in conjunction with a light-scattering detector for the determination of absolute molecular weights of dilute polymer solutions.

Universal mass detection of surfactants, lipids, carbohydrates, polymers and petroleum additives can be achieved with the Varex **laser light-scattering detector** for liquid chromatography — the LLSD MkII — from P.S. Instruments (*Reader Service No. 107*). Designed to link up to an LC separation system, the mass detector has stated applications in HPLC, gel permeation, size exclusion or supercritical fluid chromatography. The detector has, says PSI, particularly high sensitivity to compounds with specific functional groupings or chromophores. High stability and a 'noise-free' baseline performance in gradient elution analyses makes the LLSD MkII detector a useful general-purpose detector when compared with RI detectors.


P.S. Instruments' general-purpose laser light-scattering detector.

Instrumental ideas

Hewlett-Packard, in extending its modular HPLC instrument range, has introduced **titanium HPLC systems** — HP 1050 Ti Series — designed for chemical separations such as protein purification and ion chromatography that require the use of 'aggressive' mobile phases (*Reader Service No. 108*). The set-up includes a $6,850 (US) isocratic pump, $14,500 (US) gradient pump, $1,300–1,710 (US) manual injection valve option, $8,800 (US) autosampler and $6,250 (US) variable-wavelength detector. Titanium alloy is used to replace all conventional stainless steel parts in the instrument. The machine is particularly useful where formic acid in the mobile phase is required for the purification of hydrophobic proteins. Other uses include the purification of biologically-active materials when exposure to iron ions could inactivate enzymes, and where high salt concentrations are necessary to achieve sufficient column selectivity. To prevent 'cracking' associated with stress corrosion of pure titanium, the HP 1050 series contains 0.2 per cent palladium. Although the use of titanium increases prices, this, says Hewlett-Packard, can be offset against the machine's high resistance to corrosion.

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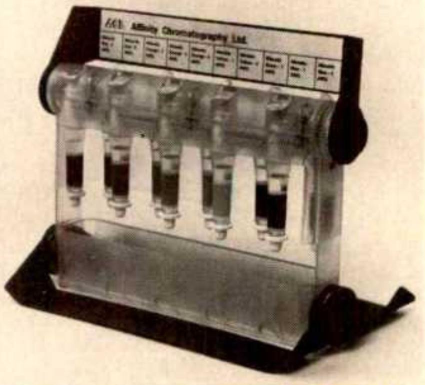
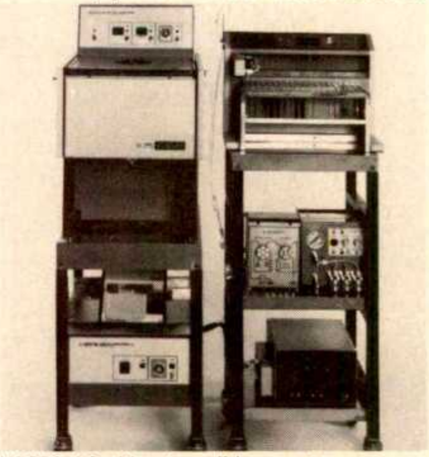
Affinity Chromatography, located in the Isle of Man, has introduced an integrated PIKSI module (Protein Identification Kit by Sorbent Isolation) for purifying proteins by **affinity chromatography** (*Reader Service No. 109*). The module contains ten mimetic affinity ligands bonded to six per cent cross-linked agarose. This arrangement is designed to maximize the purification of most proteins in just a few minutes. No knowledge of the protein's molecular weight or isoelectric point is necessary. Mimetic ligand adsorbents are available in laboratory packs or in bulk quantities.



ACL's PIKSI affinity chromatography module.

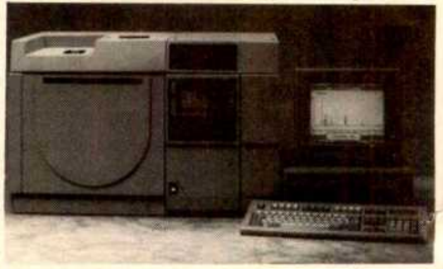
Sanki Laboratories is supplying a new modular system — the LLN-9 — for preparative/analytical **high-performance centrifugal partition chromatography** (HPCPC) (*Reader Service No. 110*). HPCPC uses liquid-liquid partition, countercurrent distribution to fractionate complex mixtures of chemical substances. Stated applications include the separation and purification of a broad range of synthetically and naturally-occurring chemical species, with particular use in the isolation of polar substances and biological materials — natural products, pharmaceuticals, biopolymers, antibodies and genetically-engineered molecules. As HPCPC does not use a solid stationary-phase support, irreversible retention of highly-retentive sample components is eliminated. Stationary-phase solvents are retained in the column by centrifugal force; this allows the use of high mobile-phase flow rates, without an appreciable loss of resolution, says Sanki.



HPCPC: LC without a solid support.

The DX-100 is an integrated, single-channel **ion chromatograph** from Waters designed to perform all types of isocratic IC separations using conductivity detection (*Reader Service No. 111*). Waters says that for analysts considering purchasing an add-on conductivity detector to an HPLC system, the dedicated DX-100 complete IC system, with no extra pumps or valves needed, is a useful alternative. The DX-100 uses advanced conductivity detection and offers 11 operating ranges from 0.01 to 1,000 μs full scale. The 4,000 psi-rated solvent-compatible pump has a 0.5–4.0 ml min^{-1} flow range with user-selectable high- and low-pressure alarms. All high-pressure flow paths are constructed of PEEK (polyetheretherketone) components for compatibility with the acids and bases used in IC. The £9,550 (UK) DX-100 IC system can be equipped with OmniPac and IonPac columns.

The PU4410 is the latest all-purpose **gas chromatograph** from Philips that is suitable for both capillary analyses and conventional packed-column chromatography (*Reader Service No. 112*). As the PU4410 is configured for increased throughput and ease of use, parameters are entered by a membrane keyboard, which is designed to guide the user through the process. Operation is simplified still further with the stopwatch, flow calculator, auto-start and parameter-locking facility. Included in the package are two capillary injectors — one split-splitless, the other a programmable thermal vapouriser injector. Other injectors are available for all types of capillary, 530 μ and packed-column studies. Five types of detector are offered, optimized, says Philips, for capillary chromatography and offering fast response times and small dead volumes. In need of help, well Philips offers a chromatography customization service to cater for all user needs. □



PU4410 GC system suits both capillary and packed-column analyses.

These notes are compiled by Diane Gershon from information provided by the manufacturers. To obtain further details, use the reader service card bound inside the journal. Prices quoted are sometimes nominal, and apply only within the country indicated.

GUIDE TO AUTHORS

Please follow these guidelines so that your manuscript may be handled expeditiously.

Nature is an international journal covering all the sciences. Contributors should therefore bear in mind those readers for whom English is a second language and those who work in other fields. Please write clearly and simply, avoiding unnecessary technical terminology. *Nature*'s staff will edit manuscripts to those ends if necessary. Contributors should check their proofs carefully.

Because of the competition for space, many of the papers submitted for publication cannot be accepted. For this reason, and because brevity is a great assistance to readers, papers should be as brief as is consistent with intelligibility. Please note that one printed page of *Nature*, without diagrams or other interruptions of the text, has fewer than 1,300 words.

CATEGORIES OF PAPER

Review Articles survey recent developments in a field. Most are commissioned, but suggestions are welcome in the form of a one-page synopsis addressed to the Reviews Coordinator. Length is negotiable in advance but must not exceed six pages of *Nature*.

Articles are research reports whose conclusions are of general interest and which are sufficiently rounded to be a substantial advance in understanding. They should not have more than 3,000 words of text or more than six display items (figures and tables) and should not occupy more than five pages of *Nature*.

Articles should be accompanied by a heading of 50 – 80 words written to advertise their contents in general terms, to which editors will pay particular attention. A heading (printed in italic type) is not an abstract and should not usually contain numbers or measurements. The study should be introduced in more detail in the first two or three paragraphs, which should also briefly summarize its results and implications.

Articles may contain a few subheadings of two or three words. The meaning of the text should not depend on the subheadings, whose function is to break up the text and to point to what follows. There should be fewer than 50 references.

Letters to Nature are short reports of outstanding novel findings whose implications are general and important enough to be of interest to those outside the field. Letters should not have more than 1,000 words of text or more than four display items and should not occupy more than two pages of *Nature*. The first paragraph should describe, in not more than 150 words, the origins and chief conclusions of the study. Letters should not have subheadings or more than 30 references.

Commentary articles deal with issues in, or arising from, research that are also of interest to readers outside research. Some are commissioned, most are unsolicited. They are normally between one and four pages of *Nature* in length.

News and Views articles are intended to inform nonspecialist readers about a recently published advance. Suggestions should be made to the News and Views Editor. Illustrations are welcome. Proposals for meeting reports should be agreed in advance.

Scientific Correspondence is for the discussion of scientific matters, including contributions published in *Nature*. Priority is given to contributions of less than 500 words and five references. Figures are welcome.

GENERAL

All contributions submitted for publication in *Nature* should conform with these rules.

Manuscripts should be typed, double-spaced, with a good-quality printer, on one side of the paper only. Four copies are required, each accompanied by lettered artwork. Four copies of half-tones should be included. Reference lists, figure legends and so on should be on separate sheets, all of which should be double-spaced and numbered. Copies of relevant manuscripts in press or submitted for publication elsewhere should be included, clearly marked as such. Revised and resubmitted manuscripts should also be clearly marked as such and labelled with their reference numbers.

Titles should say what the paper is about with the minimum of technical terminology and in fewer than 80 characters. Authors should avoid active verbs, numerical values, abbreviations and punctuation and should include one or two key words for indexing purposes.

Artwork should be marked individually and clearly with the author's name, figure number and, when known, manuscript reference number. Original artwork should be unlettered. Ideally, no figure should be larger than 28 by 22 cm. Figures with several parts are permitted only if the parts are closely related, either experimentally or logically. Suggestions for cover illustrations, with captions, are welcome. Original artwork (and one copy of the manuscript) will be returned when a manuscript cannot be published.

Colour Artwork. A charge of £500 a page is made for 4-colour figures. Inability to meet these costs will not prevent the publication of essential colour figures if the circumstances are explained.

Figure legends must not exceed 300 words and ideally should be much shorter, and should use telegraphic form wherever possible. The figure should be described first, then, briefly, the method. Reference to a method described elsewhere is preferable to a full description. Methods should not be described in detail in the text.

References should be numbered sequentially as they appear in the text, followed by those in tables and finally those in the figure legends. Reference numbers apply only to papers published or in the press, and a different number should be given to each paper cited. All other forms of reference (including unrefereed abstracts) should be included in the text as personal communication, manuscript in preparation or preprint (with number and institution where appropriate). Text should not be included in the reference list. References should be abbreviated according to the *World List of Scientific Periodicals* (fourth edition, Butterworth, London, 1963 – 65). The first and last page numbers should be cited. Reference to books should clearly indicate the publisher and the date and place of publication.

Abbreviations, symbols, units and Greek letters should be identified the first time they are used. Acronyms should be avoided as much as possible and, when used, defined. Editors will shorten words if necessary. In case of doubt, SI units should be used.

Footnotes should not be used except for changed addresses or to identify the corresponding author if different from the first-named.

Acknowledgements should be brief. Grant numbers and contribution numbers are not allowed.

***Submission.** All manuscripts may be submitted to either London or Washington. They should not be addressed to editors by name. Manuscripts or proofs sent by air courier to London should be declared as 'manuscripts' and 'value $5' to prevent the imposition of import duty and value-added tax (VAT).*

Nature Classified

LONDON Julie Skeet, 4 Little Essex St, London WC2R 3LF. Telephone: 071 872 0102. Fax: 071 240 2408.

NEW YORK – Marianne Ettisch, 65 Bleecker Street, New York, NY 10012. Telephone: (212) 477 9625. Fax: (212) 505 1364.

PARIS Clare Newell, BP No 804, 75828 Paris, Cedex 17. Tel: (1) 40 53 03 39. Fax: (1) 42 67 64 99.

MUNICH – Sabine Fürst, Sandstrasse 41 8000 München 2. Telephone: (089) 52 40 82. Fax: (089) 5 23 22 22.

TOKYO – Phillip Hamill, Shin-Mitsuke Building (4F), 3–6 Ichigaya Tamachi, Shinjuku-ku, Tokyo 162. Telephone: (03) 267 8751. Fax: (03) 267 8746.

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CANCELLATIONS MUST BE RECEIVED NO LATER THAN 5PM ON THURSDAYS PRIOR TO ISSUE DATE.

ASSISTANT PROFESSOR, MICROBIOLOGY

The Department of Microbiology and Immunology, University of North Dakota School of Medicine invites applications for a tenure track position at the rank of assistant professor. Candidates should have a PhD and postdoctoral experience. The appointee is expected to develop a competitive research program, participate in the teaching of medical and general microbiology and develop a course in his/her specialty. Although research involving a molecular approach to microbial pathogenesis, microbial biochemistry/physiology or environmental microbiology is preferred, other research areas will be considered. Review of applications will begin 1, August 1990.

Applicants are to provide a letter of application, curriculum vitae, a statement of research with representative publications and the names, addresses and phone numbers of three references from whom the applicant has requested letters to: **Dr. James J. Kelleher, Chairman, Department of Microbiology and Immunology, School of Medicine, University of North Dakota, Grand Forks, ND 58202.**

UND is an Equal Opportunity/Affirmative Action Employer.

(NW5496)A

ROYAL FREE HOSPITAL SCHOOL OF MEDICINE
(University of London)
DEPARTMENT OF IMMUNOLOGY

Research Assistant

Research Assistant is required on a Medical Research Council supported grant for three years in order to investigate immunological defects in HIV infections. The technical skills necessary for this position include isolations of lymphoid populations by fluorescence cytometry and depletion techniques, investigation of lymphocytes by immunofluorescence and the analysis of the functional performance of cells taken from HIV infected individuals. Further skills in HIV viral isolation would be an advantage.

Salary on Research Scale 1B plus £1,767 London Allowance.

Application by curriculum vitae (2 copies) plus names and addresses of 2 referees to the **School Office, RFHSM, Rowland Hill Street, London NW3 2PF (tel: 071-794 0500 ext 4262).** Please quote ref: IMM/RA/113. Closing date 20 July 1990. (4058)A

Glaxo IMB

GLAXO INSTITUTE FOR MOLECULAR BIOLOGY

The Geneva molecular biology research institute of one of the world's most successful healthcare groups is recruiting a

CELLULAR NEUROBIOLOGIST

The successful candidate will join a highly motivated team of 30 molecular and cellular biologists and biochemists studying the structure/activity relationships of neurotransmitter receptors, and factors involved in nerve cell proliferation and differentiation.

The candidate is expected to have a PhD, and several years of postdoctoral research experience in neuro-cell biology with, if possible, experience in cell line Immortalization. The candidate's experience should be demonstrated by a significant list of publications.

The Glaxo Institute for Molecular Biology carries out long term research to support the company-wide effort to discover effective new medicines. It provides a dynamic research environment, and encourages the publication of scientific results as well as collaborative projects with academic groups.

We offer an excellent salary and benefits package and opportunities for career development in one of the most attractive parts of Europe.

If you are interested in this position, please send your Curriculum Vitae with a list of publications and the names of three referees to : Rita Gloor, Personnel Manager, GLAXO IMB S.A., 46, route des Acacias, 1211 Geneva 24, Switzerland.

(W7499)A

新薬の探索・開発研究に意欲ある研究者求む

ファイザー製薬は世界の総合製薬企業ファイザーグループの重要な一員として、新薬の研究から生産・販売に至るまで日本で積極的に活動しています。

その研究活動の中心は名古屋市の南35㎞にあります。ここにはファイザー名古屋中央研究所と新薬開発センターの２つの研究所があり、新薬の探索から開発まで幅広く研究しています。

ファイザー製薬は日本での研究をさらに拡大・強化するために、以下の分野で博士の学位を有する人または同等の能力・経験のある独創的研究者を求めています。また国内外で博士研究員または企業での研究経験のある人、英語に堪能な人は特に歓迎します。

探索研究——ファイザー名古屋中央研究所

●生物科学部門

生化学・免疫学

サイトカイン、細胞表面受容体、炎症細胞の活性化等に研究経験のある人。炎症における種々のメディエーターの役割を解明する評価系を考案し実施する知識と経験のある人。

薬理学

種々の炎症に関する動物モデルに研究経験のある人。自己免疫、関節炎等の動物モデルの開発、一般薬理、細胞免疫に知識のあることが望ましい。

●合成部門

有機合成化学

有機合成反応および有機天然物合成の分野で研究経験豊かな人。生理活性物質のデザインとその合成の実施。薬物の生物学的側面について強い関心があることが望ましい。

●微生物部門

発酵・微生物学

微生物の分類、および微生物の発酵生産能力を制御することに研究経験のある人。

生化学・分子生物学・薬理学

疾病の発症機構の中に標的を選び出し、免疫学や分子生物学等の原理で評価系を確立することに必要な知識・経験のある人。

天然物化学

微生物由来の生理活性物質の単離・構造決定に経験豊かな人。

開発研究——新薬開発センター、医薬開発統括部

●製剤研究

経口および非経口製剤の処方研究、ＤＤＳ手法を用いた研究に経験豊かな人。

●薬物動態研究

薬物の代謝研究、薬動力学、統計解析に理解が深い人。

●薬理研究

炎症・免疫領域の種々の動物モデルや免疫担当細胞を用いる薬理・生化学。循環系、体液調節系、中枢神経系領域の薬理学、電気生理学および行動薬理学。

●細菌学的研究

各種抗菌剤の薬効評価およびその作用機序の研究。

●安全評価研究

動物での安全性評価研究、特に組織病理学に理解が深い人。

●臨床開発研究

循環器、免疫、炎症等に関する広範囲な薬剤の臨床開発を大学病院等医療機関と共同で推進する。関連各分野の基礎または臨床薬理の知識と臨床開発経験のある人。

応募者は履歴書、研究歴（研究業績・論文リスト）等を下記あて郵送下さい。秘密は厳守します。

〒163 東京都新宿区西新宿２丁目１－１
私書箱226
ファイザー製薬株式会社
取締役人事統括部長　高 松 健 二
TEL.03(344)4717

(W7501)A

Pfizer
ファイザー製薬株式会社
ファイザー
ファイザー名古屋中央研究所　新薬開発センター

MRC AIDS DIRECTED PROGRAMME

Research Scientists

Celltech's gene cloning and purification development teams have a collaborative research programme to express HIV-1 and SIV envelope proteins (gp120) in mammalian cells and to investigate the role of envelope transmembrane domains in the oligomerisation of gp160.

We have positions available on this programme for two post-doctoral scientists, initially for two years. If your experience and interests include expression, purification and characterisation of recombinant proteins – with specific reference to virus entry – this is your opportunity to join some of the UK's most exciting medical research projects. The research is supported by the MRC; you will work in a multi-disciplinary environment and enjoy extensive access to the MRC ADP network of academic research workers.

Cell/Molecular Biologist

You will generate stable cell lines expressing recombinant env proteins; experience in the HIV/SIV field would be desirable but is not essential. Ref: 449.

Protein Biochemist

You will need experience of protein purification, characterisation and assay development. Previous experience with viral structural proteins would be advantageous. Ref: 450.

Both positions offer highly competitive salaries, supported by comprehensive benefits packages.

Please write, with full details of your qualifications and experience, to The Recruitment Officer, Personnel Department, Celltech Limited, 216 Bath Road, Slough, Berkshire SL1 4EN, quoting the appropriate reference number.

(4100)A

CELLTECH

UNIVERSITY OF CAMBRIDGE

RESEARCH ASSISTANT

IN BIOCHEMISTRY & HUMAN MOLECULAR GENETICS

Applications are invited from graduates in biochemistry or molecular biology for a position funded for up to 4 years by the Wellcome Trust. The research will principally involve the characterization of the molecular defect in families infected by inherited disorders of metabolism.

It is planned to investigate the enzymatic defects at the level of protein and gene and to determine the precise effects of mutations by analysis of the variant proteins in expression systems. The research will involve biochemical analysis and molecular biology technology.

The salary will be in the range of £9196 to £12381 (under review).

Applicants should send 3 copies of their curriculum vitae and request further particulars from: **Professor T. M. Cox, Department of Medicine, Level 5, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ.**

The University follows an equal opportunities policy. (4084)A

JOHN INNES INSTITUTE

DEPARTMENT OF APPLIED GENETICS

A Scientific Officer/Higher Scientific Officer is required to join a group exploiting molecular techniques to study the population and evolutionary genetics of nitrogen fixing bacteria. The person appointed will assist in the isolation, culture and characterization of the bacteria and in analyses of particular DNA sequences.

Candidates should have a degree in an appropriate subject (in particular genetics or molecular biology) and for appointment to the higher level candidates should have at least five years post qualifying experience.

Salary at Scientific Officer level will be on the scale £9,131 to £12,223 and at Higher Scientific Officer level £10,678 to £14,909. Non-contributory superannuation.

Applications with full particulars and CV together with the names of two referees should be sent to the **Personnel Officer, John Innes Institute, John Innes Centre for Plant Science Research, Colney Lane, Norwich NR4 7UH.**

Closing date for applications 3rd August 1990 quoting reference AG/433.

The John Innes Institute is an Equal Opportunities Employer and is associated with the AFRS Institute of Plant Science Research.

(4061)A

MEDICAL RESEARCH COUNCIL
NATIONAL INSTITUTE FOR MEDICAL RESEARCH

Applications are invited for an

INDEPENDENT SCIENTIFIC POSITION

(five-year)

within a newly-established molecular immunology team at the Institute with special interest in thymus development, T-cell differentiation and tolerance induction. The candidate will be expected to establish a research programme in one of the above topics and therefore experience in cellular and/or molecular immunology research is essential.

The position is available immediately. The salary will be in the range £12,381 to £20,469 (starting salary dependent on experience) plus £1,767 per annum London Allowance. MRC Pension Scheme option.

Applications including a curriculum vitae, list of publications and the names and addresses of two professional referees should be sent to **Mr C R Russell, Administrative Manager, National Institute for Medical Research, The Ridgeway, Mill Hill, London, NW7 1AA**, not later than 3rd August 1990 *quoting reference number GSE/0250.* Informal enquiries about the nature of the work may be made to Dr Dimitris Kioussis on 081 959 3666 extension 2113.

The MRC is an Equal Opportunities Employer. (4106)A

MRC
Medical Research Council

University College London

Department of Geological Sciences

Postgraduate Unit of Micropalaeontology

LECTURESHIP IN PALYNOLOGY

Applications are invited from active research workers with interests in the fields of palynology, palynostratigraphy and palynofacies who will complement the scientific activities of the Department (which particulary concern post-Palaeozoic sequences) in addition to developing their own area of speciality; an interest in and experience with image analysis technology will be advantageous. The successful candidate will be expected to supervise research students in the Micropalaeontology Unit, participate in the M.Sc. Micropalaeontology Advanced Course, and contribute to Departmental undergraduate teaching. The appointment will be amde from 1 October 1990.

Salary within range £12,225–£22,236 p.a. inclusive according to age and experience.

Further information can be obtained from **Dr. A.R. Lord, Departmental Administrator, Department of Geological Sciences, University College London, LONDON WC1E 6BT.** Tel: 01-387 7050 ext. 7131. The closing date for applications is Friday 27 July 1990. *Equal Opportunities Employer.* (4055)A

5-HT Research

Two stimulating and varied opportunities

The formation of SmithKline Beecham has created a powerful force in the pharmaceutical world; a company with established global success to build on and now better equipped than ever to invest in the research and development of a substantial drug portfolio.

The climate is one of progress and change, thus it is an ideal time to join us. We are currently seeking scientists to consolidate our existing team of highly-innovative researchers in the 5-HT area.

Postdoctoral Fellow in Chemistry

This position, which is tenable for 2 years, offers an excellent opportunity for acquiring synthetic and medicinal chemistry experience in a major drug discovery area. Applicants should have a PhD with experience in heterocyclic chemistry and an interest in rational drug design. Proven technical and creative abilities as well as good communication skills are essential. Ref: 28/90.

Graduate Pharmacologist

We require a graduate to design and carry out vascular and biochemical studies, including exploratory research and screening. Applicants should have a good degree in Pharmacology or Biochemistry and some experience in Cardiovascular and Receptor Pharmacology. Experience in the use of computers for data analysis would be an advantage. Ref: 26/90.

We offer good career development opportunities in a stimulating work environment and excellent research facilities. In addition to a competitive salary, other benefits include a performance-related bonus scheme, free life assurance, medical insurance scheme, flexible working hours and subsidised sports, social and canteen facilities. Relocation assistance will be given where appropriate.

Please send your cv, quoting the appropriate reference, to Vance Frisch, Personnel Officer, SmithKline Beecham Pharmaceuticals, The Frythe, Welwyn, Herts AL6 9AR.

SB

SmithKline Beecham
Pharmaceuticals

(4077)A

ST GEORGE'S HOSPITAL MEDICAL SCHOOL
(University of London)

MOLECULAR BIOLOGIST

A post-doctoral position funded for 3 years is available in a group interested in the cellular and molecular biology of bone cells. The project involves an analysis of regulatory mechanisms in osteoclastic development and function.

Salary on the 1A scale up to £18,432 per annum including London Allowance. To apply please send curriculum vitae and names of two referees to: **Professor T J Chambers, Department of Histopathology, St George's Hospital Medical School, Cranmer Terrace, London SW17 0RE**, 081 767 6954.

(4086)A

POSTDOCTORAL POSITION

Applications are invited for a postdoctoral position (2 years) in a research group studying genetics and regulation of virulence-associated properties in bacteria (*e.g. E coli* pili-adhesins, enterotoxins; for recent publications see: *Mol. Microbiol.* **3**, (1989) 1557. *Nature* **344**, (1990) 682).

PhD in biological sciences and experience in molecular genetics and biochemistry desirable.

Applicants should send curriculum vitae, statement of research interest, and a list of names and addresses of at least three references to:

Dr Bernt Eric Uhlin,
Department of Microbiology,
University of Umeå,
S-901 87 Umeå, Sweden

(W7517)A

FACULTY POSITIONS

DEPARTMENT OF BIOCHEMISTRY AND MOLECULAR BIOLOGY

THE UNIVERSITY OF CHICAGO

The Department of Biochemistry and Molecular Biology at The University of Chicago wishes to invite applications and nominations for several tenure-track positions to be filled in the 1990–1991 academic year. Candidates should have a doctoral degree, significant postdoctoral research experience, a strong record of research accomplishment, and demonstrable potential for the development of an independent research career in an academic setting. Although we are seeking individuals with research interests in any area of modern biochemistry and molecular biology, we are particularly emphasizing recruitment of individuals with research interests in nucleic acid biochemistry, protein-nucleic acid interactions, membrane and cell biochemistry, and structural biology, including magnetic resonance and molecular dynamics.

Applications should include a *Curriculum vitae*, a list of publications, a summary of current research activities and future research plans, and the names of at least four individiuals from whom letters of reference may be solicited. While we have no formal deadline, we would appreciate receiving applications by September 15, 1990. Please send applications or nominations to:

Marvin W. Makinen
Department of Biochemistry and Molecular Biology
The University of Chicago
Chicago, Illinois 60637

The University of Chicago is an affirmative action/equal opportunity employer.

(NW5500)A

British Antarctic Survey

HEAD OF UPPER ATMOSPHERIC SCIENCES DIVISION

CAMBRIDGE **SALARY UP TO £30,001**

The British Antarctic Survey initiates and conducts programmes of scientific research in Antarctica and its surrounding oceans and atmosphere.

The Head of Division has responsibilities for the planning, co-ordination and management of scientific research in Upper Atmospheric Science.

Duties include project planning, including aspects of financial, staff, technical and field operations management, as well as performance and productivity evaluation, control of allocated funds, recruitment, training and deployment of staff in the UK and Antarctica. The Head of Division will have direct responsibility for 27 scientists.

The Head of Division is expected to promote collaborative research with other institutions both nationally and internationally. An important element is to contribute to general policy development and administration of BAS. Current research programmes of the Division include energy flow and dissipation in geospace (involving Advanced Ionospheric Sounder and Anglo-American HF backscatter radar system for conjugate experiments, and future development of Automated Geophysical Observatories) and radio wave generation and propagation (ground and satellite experimental studies and theoretical modelling of wave-particle interactions, Whistler mode propagation, etc). The successful candidate will have a high international reputation in an appropriate field of upper atmospheric science and a proven track record of research management. Antarctic experience would be desirable.

The post is at Grade 6 level within the range £23,039 to £30,001 with further increments to £35,436 depending on performance. A non-contributory pension scheme is available.

Application forms are available from Mrs L. Nowlan, Natural Environment Research Council, Polaris House, North Star Avenue, Swindon, Wiltshire SN2 1EU. Telephone 0793 411531. The British Antarctic Survey is a component institute of the Natural Environment Research Council.

The closing date for applications is 26th July 1990.

Natural
Environment
Research
Council

(4102)A

University of Manchester
Department of Physiological Sciences
RESEARCH ASSISTANT

Applications are invited from recent or prospective graduates in biological sciences, biochemistry or a related subject, to work on molecular biological aspects of neuropsychiatric diseases. The post is available from October 1st, 1990 for 3 years in the first instance. Practical experience in basic molecular biology techniques would be an advantage. The appointee will be encouraged to study for a higher degree. Salary will be in the range £9,816 – £12,381 p.a. (under review) depending on qualifications and experience.

Please apply in writing, enclosing a full CV, to **Professor F. Owen, Department of Physiological Sciences, Stopford Building, the University, Manchester M13 9PT.** Closing date: August 17th 1990.

The University is an Equal Opportunity Employer. (4069)A

Postdoctoral Position
Biochemistry Department
University of Illinois at Chicago

A funded postdoctoral position is available to study transcriptional regulation of lipid metabolism in Drosophila. Candidates should have a recent Ph.D. and a background in molecualr biology or genetics.

Please contact Dr. Hoshizaki at 312/996-5112 and send résumé with the names of three references to:

Dr. Deborah K. Hoshizaki
Biochemistry Department (M/C 536)
University of Illinois at Chicago
1853 W. Polk St., Rm. A-312 CMW
Chicago, IL. 60612. (NW5507)A

POSTDOCTORAL POSITIONS

Available immediately at The Biomedical Research Centre on The University of British Columbia campus:

With Dr. Reudi Aebersold in:

1. Analytical Protein Chemistry: Development of improved technologies for protein sequence analysis.
2. Biochemistry/Molecular Biology: Characterization of protein kinases and their substrates in T lymphocytes using biochemical and molecular genetic technologies.

With Dr. Ian Clark-Lewis in:

1. Protein-Structure Function: Structure-function analysis of protein growth factors and cytokines using cloning, and chemical (peptide) synthesis.
2. Engineering of cytokine receptors: Cloning, mutagenesis and mammalian expression, purification, structural and functional analysis.

Applicants should send a curriculum vitae and the names, addresses and phone numbers of 3 referees to: **Dr. Aebersold or Dr. Clark-Lewis:**

The Biomedical Research Centre
2222 Health Sciences Mall
The University of British Columbia
Vancouver, British Columbia
V6T 1W5
Canada (NW5505)A

University of Düsseldorf, Neurobiology Laboratory, Department of Neurology
MOLECULAR/CELL BIOLOGISTS and PROTEIN BIOCHEMIST

Applications are invited for postdoctoral research associates to join the Molecular Neurobiology Research Group working on the cloning, expression and regulation of genes relevant to nerve regeneration and on the biochemical characterization and function of glial growth factors in the central nervous system. Salary according to age and qualification (65,000-75,000 DM p.a.).

Send letter of application with full CV, summary of research interest and names of two referees to: **H. W. Müller, PhD, Department of Neurology, University of Düsseldorf, Moorenstr. 5, D-4000 Düsseldorf, Germany.** (W7516)A

INSTITUTE OF OPHTHALMOLOGY
(University of London)
Judd Street, London WC1H 9QS
Tel: 071-387 9621 ext 253

RESEARCH ASSISTANT

required to join a group based in the Department of Pathology located at Cayton Street, London EC1, working on autoimmune mechanisms in ocular disease. Previous experience in immunology helpful and training can be given in molecular biology. The ability to work as part of a team is essential. Salary in the range £11,583 – £14,148 (including LA) according to age and qualifications. The appointment will be for three years in the first instance. Candidates should send a cv and the names of three referees to **Miss Audrey Jones, Assistant Secretary,** at the above address. Closing date 19 July 1990.
(4054)A

Parke Davis Research Unit, Cambridge.

DIRECTOR: DR JOHN HUGHES

RESEARCH ASSOCIATES IN PHARMACOLOGY

This unique Unit is developing new generation drugs for the treatment of nervous and neurohormonal disorders. Our basic research interests centre on the function and mechanism of action of hormones and neurotransmitters at the behavioural, cellular and molecular levels with particular reference to peptides. We have a number of strong research programmes involving permanent staff, Ph.D. students and Post-doctoral Fellows. Collaborative projects with several University, Research Council and Hospital research groups are also in progress.

As part of an expansion of our facilities in Cambridge, we have vacancies for young graduate pharmacologists. Applicants should be recent graduates in pharmacology or a closely related subject. Experience of working in a research laboratory in industry would be an advantage. The positions, which are available immediately, carry a starting salary in the range £11,462 to £13,111 on a scale rising to £23,167. Starting salary will be dependant on qualifications and experience. Other benefits include lunch allowance, Christmas bonus, contributing Pension Scheme, life assurance and relocation assistance where appropriate.

Applications should be accompanied by a full up-to-date curriculum vitae, together with the names of two referees, and sent to:

Dr. Chris Bruton,
Head of Administration & Personnel,
Parke Davis Research Unit,
Hills Road,
Cambridge CB2 2QB

to arrive as soon as possible and in any event by 27th July 1990.

WARNER
LAMBERT

(4107)A

The University of Queensland

Equal Opportunity in Employment is University Policy

Chair of Geology and Mineralogy

Applications are invited for the Chair of Geology and Mineralogy from geoscientists of high academic standing with substantial research achievements. The appointee will be strongly committed to education in geology at all levels; have considerable teaching and administrative experience, and a demonstrated capacity for collaboration with industry and the attraction of external funds.

It is expected that the appointee will assume significant administrative responsibilities, including the Department Headship for at least the first five years of appointment.

In addition to the advertised chair, the Department has 14 full-time teaching staff positions, including the **Dorothy Hill Chair of Palaeontology and Stratigraphy**, and 21 research and support staff. The Department has excellent research facilities for a range of disciplines including petrology, elemental and stable isotopic geochemistry, palaeontology, sedimentology, and geophysics; a museum and a first-class library. The **W H Bryan Mining Research Unit**, housed within Geology and Mineralogy, is a recent joint venture with the Department of Mining and Metallurgical Engineering.

The Department offers 3 and 4-year (Honours) courses leading to the BSc degree in Geology, 4-year courses leading to Bachelor of Applied Science (Geophysics) and Bachelor of Bachelor of Applied Science (Geology), and coursework leading to Master of Scientific Studies. There are currently 41 postgraduate students, of whom 21 are PhD or research MSc candidates.

The salary for a Professor is A$67,812 per annum. **Ref. No:** 23990. **Closing Date:** 31 August 1990.

Further information is available from the Head of Department Dr G R Orme on (07) 377 3910.

Application forms and further details concerning method of application are available from the Appointments Officer, Association of Commonwealth Universities, 36 Gordon Square, London, WC1H OPF. (W7445)A

COMMONWEALTH SECRETARIAT

SCIENCE ADVISER

Applications are invited for the post of Science Adviser to the Commonwealth Secretary-General. The Science Adviser is also Secretary of the Commonwealth Science Council (CSC) which aims to enhance scientific and technological capability in Commonwealth countries by enabling them to undertake research and training to solve development problems. The Science Adviser/Secretary CSC who leads a small team of scientists and supporting staff advises the Secretary-General on the scientific and technological implications of the Secretariat's programmes in the context of global issues and trends, the improvement of indigenous technologies and the identification of areas of innovation. He/she will be responsible to the CSC for the planning, management and control of CSC programmes, budgetary and staff matters. Considerable travel may be involved. Applicants, who must be Commonwealth citizens, should have a doctoral-level degree or equivalent qualification in science or engineering, with experience in post-doctoral research and the planning and management of applied science and technology. Applicants must demonstrate capacity for innovative planning and independent action, should have a general awareness of the emerging scenarios in science, technology and environment, and be conversant with the broad lines of science policy and research, especially in the Commonwealth, sources of expertise and of funding. The Adviser/Secretary CSC is required to function effectively in a multi-cultural environment. As an equal opportunity employer, the Secretariat specially welcomes applications from suitably qualified women.

Appointment is for two to three years initially. Salary range £30,228-£35,448 pa gross plus for UK resident 12 ½ per cent salary and for overseas appointee expatriation and other allowances. Job description and further details available on request. Please send detailed CV, quoting ref ON 37/90, with names and addresses of three referees by 7 September 1990 to the **Chief Personnel Officer, Commonwealth Secretariat, Marlborough House, Pall Mall, London SW1Y 5HX. Telephone No 071-839-3411 extension 8153 or 8134, fax 071-930-0827.**

AN EQUAL OPPORTUNITY EMPLOYER (4080)A

Graduate Scientist

The National Radiological Protection Board is the major point of reference for radiological protection in the UK, providing advisory and technical services to users of radioactive materials and those with responsibilities for Radiological Protection in industrial, medical, research and teaching situations.

A vacancy exists in the Biomedical Effects Department for a postdoctoral or graduate research scientist to carry out work on the placental transfer and fetal accumulation of radionuclides and their effects, as part of the Department's evaluation of occupational and environmental exposure to radioactive materials.

The successful candidate will carry out an experimental programme and contribute to the development of models to assess *in utero* radiation doses and carcinogenic risk. He/she may be involved in more general studies of the behaviour of radionuclides in the body.

Applicants should possess research experience in animal physiology with an interest in, and knowledge of, embryology and developmental biology.

The appointment will be in the grade of Higher Scientific Officer with a starting salary within the range £10,875-£16,590pa depending on age, qualifications and experience.

Application forms can be obtained from Establishments and Personnel Branch, National Radiological Protection Board, Chilton, Didcot, Oxon. OX11 0RQ, or telephone Abingdon (0235) 831600 exts. 2553/2548, quoting ref: PER/A324. Closing date for receipt of completed application forms: 20th July 1990. (4083)A

NRPB

National Radiological Protection Board

THE ROYAL VETERINARY COLLEGE

(University of London)

POSTDOCTORAL RESEARCH ASSISTANTS

2 POSTS

Department of Veterinary Basic Sciences

Applications are invited for postdoctoral research assistants to work on each of the following projects:

1. 'Effects of environmental temperature on the development of tissue cellularity in the Atlantic Salmon'. Experience in tissue culture techniques would be an advantage.
2. 'The determination of muscle fibre number in the very early critical stages of gestation'. Experience in immunocytochemistry and molecular biology would be useful.

The appointments are grant-funded by the NERC and AFRC and are for 3 years. Both appointments are immediately available.

Salary is in the range £13,166 - £19,932 including London Allowance. Eligibility for the Universities' Superannuation Scheme.

Applications including C.V. and the names of three referees should be sent to the **Assistant Personnel Officer, The Royal Veterinary College, Royal College Street, London NW1 0TU. Closing date for application is 26 July 1990.** (4089)A

POSTDOCTORAL POSITION

A postdoctoral position is immediately available for recent or experienced PhD (genetics, biochemistry or equivalent training) to study th molecular basis of schizophrenia using linkage analysis. Experience in current molecular genetic techniques is prerequisite.

Send CV and three references to **Dr. N.P. Anagnou, Dept. Basic Sciences, University of Crete School of Medicine, 711 10 Heraklion, Crete, Greece.** FAX # (081) 233-669. (W7500)A

Novo Nordisk

Molecular Biologist

The CNS Division of Novo Nordisk has a new position available in the newly established Molecular Neurobiology Task Force.

The successful candidate will join a team of scientists engaged in basic research and drug discovery programs directed towards the identification of novel therapeutic approaches to CNS disorders such as epilepsy, ischaemia, anxiety and schizophrenia.

The candidate will concentrate on the molecular aspects of the research areas and will become a member of the Molecular Biology Task Group in the CNS Division.

Qualified applicants are expected to be at the Ph.D. level with experience in neuroscience. Familiarity with neuroanatomy, histology and histochemical techniques, especially in situ hybridization is preferred. Experience with blotting techniques, autoradiography and cell culturing is desirable.

Novo Nordisk CNS Division is located approximately 5 miles from Copenhagen.

Interested candidates should submit their curriculum vitae and description of research experience. Please mark the application "Histo.496".

Novo Nordisk A/S
Health Care Group
Krogshoejsvej 41
DK-2880 Bagsvaerd

Att.: Personnel Department

Novo Nordisk is one of the world's leading biotechnology companies. It is a major force in insulin manufacture and diabetes treatment and is the world's largest producer of industrial enzymes. The company also manufactures and markets a variety of other pharmaceutical and bioindustrial products. Headquartered in Denmark, Novo Nordisk employs more than 8.000 people in 30 countries.

(W7503)A

THE UNIVERSITY OF THE WEST INDIES
Mona, Jamaica

The University of the West Indies (UWI) is supported by fourteen English-speaking Caribbean countries. There are campuses in Jamaica, Trinidad and Tobago, and Barbados, and University centres in eight of the other contributing countries. The Mona Campus is situated in a valley near Kingston. There are, at present, 5,086 undergraduate and 586 postgraduate students on that campus.

The University has made the development of Science and Technology in the Caribbean region a priority and is encouraging the development and prosecution of major research programmes, and collaboration with governments and other institutions in the region.

The University is seeking candidates with a proven record of achievement for the following appointments, which will be on tenure or tenure track.

PROFESSOR IN THE DEPARTMENT OF BOTANY

The Botany Department has a complement of seven academic staff and trains students for the BSc, MPhil and PhD degrees. Facilities include a tissue-culture laboratory, three greenhouses, an experimental field and a scanning electron microscope. The Department can share other facilities located within two Marine Laboratories, a Biotechnology Centre, and a Reactor Centre. This provides access to additional major items of equipment. The Campus computer is an IBM 4381 mainframe. Funding for other equipment and additional recurrent is being pursued. The Science Library has 40,000 volumes and over 1,500 subscriptions.

DIRECTOR OF THE CENTRE FOR MARINE SCIENCES
(PROFESSORIAL POST)

The Marine Sciences Centre is being developed from an existing Unit which is presently under the aegis of the Department of Zoology, Faculty of Natural Sciences. It will undertake research and training taking into account the special circumstances of the Caribbean and provide a forum for information exchange on marine science related activities. The activities are mainly carried out in two Marine Laboratories; one at Port Royal on the south coast, and the other at Discovery Bay on the north coast. The Centre will benefit from the common services and facilities of the Faculty on Mona campus.

PROFESSOR IN THE DEPARTMENT OF ZOOLOGY

The Zoology Department has a complement of fourteen academic staff members. The Department trains students for the BSc, MPhil and PhD degrees; and currently there are 478 undergraduate and 32 postgraduate students enrolled. The Department is well housed and equipped.There is a marine laboratory, which includes a newly constructed aquaculture laboratory, on the south coast; and another at Discovery Bay on the north coast. Facilities include electron microscopes; other central facilities include an Analytical Laboratory, a Neutron Activation Laboratory, and an Electronics Maintenance and Design Centre. The Campus computer is an IBM 4381 mainframe. Funding for other equipment and additional recurrent is being pursued.

GENERAL

Appointees will be expected to contribute significantly to the development of their discipline and to lead major research programmes. The University will make every effort to provide the appointees with funding for postdoctoral fellowships, research assistants, research students, and for conference travel. Fund raising through grants will be encouraged. Comfortable housing will be provided at nominal rental and other attractive perquisites are offered. Consultancy is allowed within the University rules.

Interested persons are invited to apply to the **University Registrar, University of the West Indies, Mona, Kingston 7, Jamaica,** from whom further particulars are available. Applicants should submit full curriculum vitae and outline their present main field of research and programmes they would wish to pursue at UWI. The names and addresses of three referees should also be included. Further particulars of the posts are also available from the **Appointments Officer, Association of Commonwealth Universities, 36 Gordon Square, London WC1H 0PF, to whom candidates in the UK should also send one copy of their application.** (W7510)A

POSTDOCTORAL RESEARCH AT THE NATIONAL CENTER FOR TOXICOLOGICAL RESEARCH

Research efforts at NCTR, an FDA laboratory in central Arkansas, focus on testing the assumptions used in assessing risks posed by toxic chemicals emphasizing studies in biomarkers, modulators of toxicity, and extrapolation/risk assessment. Postdoctoral stipends begin at $31,750 per year. U.S. citizenship or PRA status required. Contact:

Postgraduate Research Program at NCTR,
Science/Engineering Education Division,
Oak Ridge Associated Universities,
P.O. Box 117,
Oak Ridge, TN 37831-0117,
(615) 576-3190. (NW5506)A

POSTDOCTORAL FELLOW

$A32,472-$A39,724

LABORATORY FOR MOLECULAR BIOLOGY
DIVISION OF BIOMOLECULAR ENGINEERING,
NORTH RYDE, AUSTRALIA

We have a position for a Molecular Virologist:

THE JOB: The successful applicant will be required to work on the development of an ovine viral vector system for the delivery of recombinant vaccine antigens. Experience in recombinant DNA technology, preferably in the manipulation of viral genomes and in cell transfection and the analysis of gene expression are desirable. Skills in the growth and maintenance of viruses in tissue culture would be an advantage.

THE PERSON: Applicants should possess a PhD or equivalent qualification in virology or molecular biology and should, preferably, be able to take up the appointment by 31/10/90.

CONDITIONS: Appointment will be for a term of three years with Australian Government superannuation available.

MORE INFORMATION; Prospective applicants are invited to write to or telephone Dr. G. Both on (02) 886 4888 for further information. Selection criteria are available from Ms. Maree O'Neill on the same number.

APPLICATIONS: Applications quoting reference PG1/60 should be submitted by 27/7/90. They should be framed against the selection criteria and should state relevant personal particulars including details of qualifications and experience. Applicants should nominate and provide fax numbers for at least two professional referees and address their applications to:

The Chief,
Division of Biomolecular Engineering,
Laboratory for Molecular Biology,
P.O. Box 184,
North Ryde NSW 2113, Australia.

CSIRO
AUSTRALIA

CSIRO IS AN EQUAL OPPORTUNITY EMPLOYER AND PROMOTES A SMOKE FREE ENVIRONMENT (W7505)A

PERMANENT TRACK (ASSISTANT/ASSOCIATE or FULL PROFESSOR)

position in developmental immunology and/or immunological aspects of hematopoiesis. Successful applicants will be expected to develop outstanding independent research programs which could involve for example: B or T cell ontogeny, development of T cell repertoire, or cytokine regulation of lymphocyte development. Strong interest in graduate and postdoctoral education programs required. Highly interactive, academic immunology department of ten independent laboratories located within Mayo Clinic medical complex. Excellent laboratory facilities. Startup funds are available. Candidates with proven productivity and credentials please send curriculum vitae, statement of research interests, and names of three references to **Larry R. Pease, Ph.D., Department of Immunology, Mayo Clinic, Rochester, MN 55905.** *An equal opportunity/affirmative action employer.* (NW5464)A

UNIVERSITE DE LAUSANNE

The Medical Faculty of the Lausanne's University invites applications for two positions at its institute of microbiology

Professor of Microbiology with expertise in Virology

The professor will be the head of the Institute. Duties will involve the administration of the Institute, teaching to medical students and conducting research.

Professor of medical bacteriology

The professor will be the head of the division of medical bacteriology and diagnostic. Duties involve teaching to medical students and conducting research.

The appointed Professors are expected to be able to teach in french within two years from the date of appointment.

Applications including a curriculum vitæ, a short description of research perspectives, a list of publications and five reprints of the three most important publications should be submitted to: **Professor Y. Saudan, Dean of the Faculty of Medicine, University of Lausanne, rue du Bugnon 9, CH-1005 Lausanne,** by August 31st, 1990. From whom further information can be obtained. (W7508)A

International Research Laboratories (IRL)
CIBA-GEIGY (Japan) Ltd.

IRL is one of the fundamental research organizations of CIBA-GEIGY Group, established in 1986 in Japan. The total number of research scientist is about 80 at present, and will be further increased to 130 toward 1994 in the laboratories of Bio-organics, New Materials, Analytics & Informatics and Chemistry. Now, IRL offers:

One Postdoctoral Position
in the Research Field of
Signal Transduction Mechanism with Smooth Muscle Cells

for a period of one to two years
beginning in November or December 1990

Essential qualifications are:
A Ph.D., up to 35 years old
Of any nationality, but able to communicate in English and/or Japanese

Applications are invited immediately, to include curriculum vitae, a publication list and a concise description of interested research field to:
M. Shiono, Planning & Coordination, CIBA-GEIGY (Japan) Ltd., 10-66, Miyuki-Cho, Takarazuka, Hyogo Pref. JAPAN, Tel. 0797-74-2458, Fax. 0797-74-2325

(W7502)A

University of Glasgow Department of Surgery
and
Beatson Institute for Cancer Research

LECTURER

Applications are invited for a Lectureship in the Department of Surgery, Glasgow Royal Infirmary and for the appropriate candidate there will be an associate appointment with the Beatson Institute for Cancer Research. It is expected that the successful applicant, where applicable, will hold responsibilities on both sites and will be expected to take part in collaborative research between the two institutions in the field of molecular or cell biology of solid tumours.

The department of surgery has an active clinical programme in surgical oncology and has research interests in tumour cell proliferation and targeting of therapy to metastasis.

There is at present active collaboration in the fields of metastasis (with Dr. George Birnie) and carcinogenesis (with Dr. Allan Balmain). The successful applicant is not necessarily restricted to one of these areas, but it is hoped that they will take advantage of the close association between the two departments. There are extensive modern laboratory facilities within the department of surgery which are presently being re-equipped and, when appropriate, use can be made of the established facilities at the Beatson Institute together with some research support.

This appointment is for five years initially, and the salary range will be on the University non-clinical lecturer scale £12,086-£22,311.

Initial enquiries can be made to Professor T.G. Cooke, University Department of Surgery, Royal Infirmary, Glasgow. Tel: 041-552 3535 Ext. 5429 and further information can be obtained from Dr. John Wyke, Beatson Institute for Cancer Research, Switchback Road, Bearsden, Glasgow. Tel: 041-942 9361.

Further particulars may be obtained from the **Academic Personnel Office, University of Glasgow, Glasgow, G12 8QQ,** where applications (12 copies), giving the names and addresses of not more than three referees should be lodged on or before 4th August, 1990.

In reply please quote ref. no. 7008(M). (4075)A

HANNAH RESEARCH INSTITUTE
DEPARTMENT OF BIOCHEMISTRY & MOLECULAR BIOLOGY
RESEARCH ASSISTANT

Applications are invited from post-graduate or post-doctoral biochemists to work on a two-year project funded by the British Heart Foundation to investigate mechanisms involved in the control of the expression of HMG-CoA reductase (the rate-limiting enzyme for cholesterol synthesis) by Ca^{2+}-mobilizing hormones.

Salary will be up to £10,850 (increase pending) depending on qualifications and experience. Non-contributory pension scheme. HRI/189.

PHYSICAL CHEMISTRY GROUP
RESEARCH ASSISTANT — PhD OPPORTUNITY

A vacancy exists in the Physical Chemistry Group for a physical scientist to join a team studying the structures of gels and networks produced during the coagulation of concentrated colloidal dispersions. The research will involve development and application of novel laser light scattering and rheological techniques.

The post is a fixed term appointment available for 3 years. Applicants should possess a first or upper second class honours degree in Physics, Chemistry or a related subject. The successful candidate will have the opportunity to work towards the degree of PhD at the University of Glasgow.

The post is graded Scientific Officer. Salary Scale £9,131–11,192 per annum. Non-contributory pension scheme. HRI/190.

Further particulars are available from the Secretary, Hannah Research Institute, AYR KA6 5HL, to whom applications including the names and addresses of two referees should be sent by 27 July 1990 quoting the appropriate reference.

PHYSICAL CHEMISTRY GROUP AND UNIVERSITY OF DURHAM, DEPARTMENT OF CHEMISTRY
SERC/CASE POSTGRADUATE AWARD

Chemist with an interest in solid state NMR techniques required to work on the structure of amorphous and poorly crystalline biological calcium phosphates. Candidates should have a first or upper second class honours degree and will be registered for the degree of PhD at the University of Durham. Interested applicants should contact Dr C Holt (0292 76013) or Professor R K Harris (091 3743121). (4060)A

RESEARCH SCIENTIST/ SENIOR RESEARCH SCIENTIST

GEOPHYSICAL FLUID DYNAMICS

AUS $31,525 p.a. to $45,699 p.a.

INSTITUTE OF NATURAL RESOURCES AND ENVIRONMENT

DIVISION OF ATMOSPHERIC RESEARCH, MELBOURNE, AUSTRALIA

THE FIELD: Strait flows and Coastal Current Dynamics.

THE DIVISION: The Division of Atmospheric Research has a staff of 150 including 90 professional scientists and undertakes research into physical, chemical and dynamic aspects of atmospheric phenomena and related areas.

THE JOB: To undertake research into the character of the stratified Bass Strait overflow and its consequences for the Tasman Sea circulation and non-linear coastal hydraulics generally, using laboratory and theoretical techniques. Studies of coastal systems and boundary currents affected by both stratified or Rossby-wave hydraulics are planned as an extension of this work.

THE PERSON: Applicants should possess a Ph.D. degree or equivalent in geophysical fluid dynamics, together with experience, and aptitude for laboratory studies.

CONDITIONS: Appointment will be for a term of three years with Australian Government superannuation benefits available. Salary will be commensurate with qualifications and experience.

MORE INFORMATION: Prospective applicants are invited to contact the Division by telephone (613) 586 7666, facsimile (613) 586 7600, or telex AA34463, for a copy of the job description and selection criteria. Dr. Peter Baines may be contacted for further information.

APPLICATIONS: Should be submitted by the 31st July 1990 and quote reference AT9005. They should be framed against the selection criteria and should provide relevant personal particulars, including qualifications, transcripts of tertiary results and experience. Copies of one or two relevant publications by the applicant would be desirable. Applicants should nominate at least two professional referees and address their application to:

The Chief,
CSIRO Division of Atmospheric Research,
Private Bag 1,
Mordialloc, Vic. 3195,
Australia.

CSIRO
AUSTRALIA

(W7504)A

CSIRO IS AN EQUAL OPPORTUNITY EMPLOYER
CSIRO PROMOTES A SMOKE-FREE WORK ENVIRONMENT

AT 90/03

U.K. HUMAN GENOME MAPPING PROJECT

Paediatric Research Unit
Division of Medical & Molecular Genetics
United Medical & Dental Schools of Guy's & St. Thomas's Hospitals

GUY'S CAMPUS

RESEARCH ASSISTANTS 1B

The research assistants (1B) are required to join an expanding group working on the physical mapping of the X-chromosome and on the study of mutations which causes the X-linked diseases haemophilia and muscular dystrophy. The work will be carried out under the supervision of Dr David Bentley and Professor Francesco Giannelli.

One post is available to develop and evaluate methods for automated analysis of DNA sequences, YAC and cosmid fingerprints and mutations, using fluorescent labelling technology. The other post is to assist in the development and implementation of procedures for isolating and analyzing YAC clones by both polymerase chain reaction (PCR) and hybridization-based approaches. The posts are tenable for three years.

For more information please contact Dr David Bentley on 071-955-4645.

Applications including a C.V. and names of three referees to **The Administrator, Paediatric Research Unit, Division of Medical and Molecular Genetics, 8th Floor, Guy's Tower, Guy's Hospital, LONDON SE1 9RT**, by 27th July. (4056)A

JCU JAMES COOK UNIVERSITY OF NORTH QUEENSLAND

GEOLOGY DEPARTMENT

(including National Key Centre for Economic Geology and Marine Geophysical Laboratory)

POSTGRADUATE RESEARCH TOPICS IN 1991

Applications are invited for postgraduate students to work with research teams in the following areas:

- Terrane analysis and geomorphic studies in the northern Tasman fold belt (Assoc. Professors R.A. Henderson and P.J. Stephenson)
- Structural controls on the localisation of ore deposits (Drs. T.H. Bell, S. Johnson and K. Lawrie)
- Field processes and the origin of ore deposits (Professor G.N. Phillips, Assoc. Professor R.G. Taylor, Drs. M.J. Rubenach, P. Williams and R. Myers)
- Effects of sea-level change on sedimentary facies (Professor R.M. Carter, Dr. C. Fulthorpe)
- Marine geoscience, including shelf studies and deep-ocean swathe mapping (Assoc. Professors D.P. Johnson and R.A. Henderson, Professor R.M. Carter, Mr. P. Larcombe)

Applicants should possess a high-quality honours or masters degree in geology or a related science. Australian nationals or residents are eligible for Australian Postgraduate Scholarship Awards (closing date 31 October annually). Overseas applicants from Europe and North America are eligible for Overseas Postgraduate Research Awards (closing date 28 September annually). Overseas students from most other countries may apply for Equity and Merit Scholarship Awards (closing date 31 May annually).

Further information available on individual research programs from any of the above research personnel, or the Director of Graduate Studies, Department of Geology, James Cook University, phone (077) 814536, facsimile (077) 251501.

Further details on Scholarships and method of application from **Postgraduate Admissions, James Cook University, Townsville Qld 4811, Australia,** phone (077) 814755, facsimile (077) 796371.

(W7506)A

KUWAIT INSTITUTE FOR SCIENTIFIC RESEARCH

Kuwait Institute for Scientific Research (KISR) is a nonprofit organization with a staff of more than one thousand employees engaged in applied research in the fields of environmental and earth sciences; water resources; food resources; engineering; petroleum, petrochemicals and materials and techno-economics.

KISR requires a:

MOLECULAR GENETICIST

- **Major Duties:**
  Carries out extensive research on the genetic improvement of industrial microorganisms using recombinant DNA technology; develops advanced methods for cloning, analysis of gene expression in microorganisms and DNA/RNA probes.
- **Qualifications:**
  Applicants should have a Ph.D. in Molecular Genetics or Molecular Biology with a minimum 5 years experience in recombinant DNA technology. Experience in tissue culture techniques is desirable but not essential.

KISR offers attractive tax free salaries commensurate with qualifications and experience and generous benefits that include gratuity, free furnished air-conditioned accommodation, school tuition fees for children, contribution to a savings plan, six weeks annual paid vacation, air tickets, free medical care and life insurance.

Interested applicants are requested to send their Curriculum Vitae with supporting information not later than one month from the date of this publication, to:

Director General
Kuwait Institute for Scientific Research
P.O. Box 24885 Safat
13109 Safat — KUWAIT.

(W7509)A

Senior Pharmacologist

from £19,890 pa

Ware, Herts

Glaxo is Britain's premier pharmaceutical company, with a reputation for excellence in its research.

Success has brought an expansion of our research into disorders of the gastrointestinal tract and we are looking for a recent PhD in Pharmacology to join us.

Gastrointestinal Pharmacology is one of our newer departments but is already working on a broad range of important diseases. You would join a team researching peptide hormone receptors in the GI tract.

You should have a sound background in pharmacology and, ideally, experience of research on receptor classification. We're looking for someone with drive and commitment to achieving goals, as well as the ability to innovate - new medicines don't come easily. As a key member of a project team, you should be ready to take responsibility and to put forward ideas to the whole project group.

We're asking for a lot, but we've a lot to offer in return: good salary, with performance based increases, relocation assistance where appropriate, free pension, flexible hours, subsidised restaurant and sports and social facilities on site. We have the scientific facilities for you too - well equipped laboratories, library and information resources.

Send a CV, or write quoting ref NT 100 for an application form, to: The Research Personnel Officer, Glaxo Group Research, Park Road, Ware, Herts SG12 0DP.

Glaxo Group Research

(4101)A

Molecular Biologist

(Fixed-Term Contract)

Celltech is one of Europe's leading biotechnology companies, and a pioneer in the application of molecular biology to the discovery of new pharmaceutical products. We are looking for an experienced scientist to join our gene cloning team, on a fixed-term contract lasting up to one year.

This is an ideal opportunity to apply practical molecular biology skills in a results-driven industrial context – and make a key contribution to our advanced drug discovery programme. Ideally a PhD-qualified life-scientist, you must have at least three years' sound experience in molecular biology which includes relevant DNA cloning and sequencing work. Your practical scientific skills should be balanced by adaptability, initiative and good communication skills.

A highly competitive salary will be supported by a comprehensive benefits package.

Please write, with full details of your qualifications and experience, to The Recruitment Officer, Personnel Department, Celltech Limited, 216 Bath Road, Slough, Berkshire SL1 4EN, quoting reference EFW.

(4099)A

CELLTECH

DIRECTOR HOLLINGS ONCOLOGY CENTER

MEDICAL UNIVERSITY OF SOUTH CAROLINA

The Director for a new cancer center to be established at the Medical University of South Carolina in Charleston will have a mission to develop and maintain a coordinated and multi-disciplinary center of excellence in cancer research, treatment and outreach.

The Hollings Oncology Center is a University Center on this campus and its planning and administrative oversight is by a university center planning and management group appointed by the President. Ground breaking for its separate building is in October, 1990.

The Candidate's background should include administrative experience commensurate with directing an academic department or institute and a record of outstanding achievement in clinical and research oncology.

Nominations and credentials including bibliography and curriculum vitae should be sent to **Edward, L. Hogan, M.D., Chairman, Search Committee for Director of Hollings Oncology Center, Office of the President, Medical University of South Carolina, Charleston, South Carolina, 29425.**

MEDICAL UNIVERSITY OF SOUTH CAROLINA
171 Ashley Avenue
Charleston, South Carolina 29425

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British Geological Survey

GROUNDWATER CHEMIST

A vacancy exists in the Survey's Hydrogeology Research Group based at Wallingford in Oxfordshire to join teams investigating and predicting the hydrogeochemical processes affecting groundwater quality and pollutant transport. The successful candidate will be involved in the interpretation of field and laboratory investigations of the behaviour of a variety of inorganic solutes and synthetic organic chemicals in the groundwater environment.

Applicants must be numerate and should have, or be obtaining, an honours degree in chemistry, chemical engineering, environmental sciences or geological sciences with a sound knowledge of chemistry.

The appointment will be for a period of three years with the possibility of extension.

Salary will be within the range £9,131 to £14,909 depending on experience and qualifications. Terms include a generous leave allowance and a non-contributory pension scheme.

For an application form, to be returned no later than 26th July 1990, write to:- Establishments (Recruitment), British Geological Survey, Keyworth, Nottingham NG12 5GG. Please quote reference no. SO/WL/90/2.

The Natural Environment Research Council is an equal opportunities employer.

Natural
Environment
Research
Council

(4073)A

Postdoctoral Position: Molecular Immunology

A postdoctoral position available August 1990 to study the development of the immune system. Insertional mutagenesis via homologous DNA recombination in embryonic stem cells will be employed to generate mutant mouse strains. A background in molecular biology immunology or embryology is required. Send curriculum vitae and the names of three references, with telephone numbers to: **Dr. Mark W. Moore, University of Southern California, Comprehensive Cancer Center, 1441 Eastlake Ave., Los Angeles, California 90033-0800. FAX line (213) 224-6593.** *EOE/AA.*

(NW5498)A

POSTDOCTORAL MOLECULAR BIOLOGIST

for molecular parasitology laboratory. Experience required in the production of expression libraries, cloning, sequencing and approaches to study gene regulation in trypanosomes. Submit curriculum vitae and names (with addresses) of three references to: **Dr. F. Kierszenbaum, Department of Microbiology, Michigan State University, East Lansing, MI 48824.** *MSU is an Equal Opportunity/Affirmative Action Employer.*

(NW5501)A

PORTSMOUTH POLYTECHNIC

Biophysics Laboratories
School of Biological Sciences

LECTURESHIP IN PHYSICAL BIOCHEMISTRY/MOLECULAR BIOLOGY

REF. SCI 110

Applications are invited for a temporary Lectureship in the School of Biological Sciences. The post has been made available by the award of a Royal Society Leverhulme Trust Senior Fellowship to Dr. G. G. Kneale, and is available for one year from 1 October 1990.

The successful candidate will be expected to teach on the B.Sc. (Hons) degree course in Molecular Biology, including aspects of biophysics and physical biochemistry. There will also be the opportunity to become involved with current research in the Biophysics Laboratories, and candidates with interests in protein-nucleic acid interactions would be especially welcome.

Salary will be at an appropriate point on the Lectureship scale, currently £10,827-£16,521.

Application forms and further particulars are available from the **Personnel Office, Nuffield Centre, St. Michael's Road, Portsmouth PO1 2ED. Telephone (0705) 843257.**

Closing date for applications: 19 July 1990. (4093)A

Portsmouth Polytechnic pursues a policy of equality of opportunity. Applications are particularly welcome from people with disabilities.

Faculty Position

Department of Biochemistry

Stanford University School of Medicine

Application or nominations are invited for a faculty position in the Department of Biochemistry at the level of Full Professor.

Stanford University is committed to increasing representation of women and members of minority groups on its faculty and particularly encourages applications from such candidates.

Applicants should have an established record of excellence in original research and in teaching. Candidates should submit a *curriculum vitae* including a list of publications, a description of their research interests, and names, addresses and telephone numbers of three references to: **Search Committee Chairman, Department of Biochemistry, Beckman Center B400, Stanford University School of Medicine, Stanford, CA 94305-5307.** (NW5497)A

UNIVERSITY OF EDINBURGH
FACULTY OF MEDICINE
DEPARTMENT OF ANATOMY
NON-CLINICAL LECTURESHIP IN HUMAN ANATOMY

Applications are invited for a Lectureship which is available from October 1st 1990, in the Department of Anatomy under the University Funding Committee's new academic appointment scheme. Under the terms of this scheme, which is intended to provide permanent appointments for young staff, preference will be given to candidates at an early stage in their careers. The individual appointed should ideally be clinically qualified with some experience in the teaching of topographical anatomy. Candidates should preferably be familiar also with teaching of Histology, Neuroanatomy and Embryology and be actively involved in anatomically orientated, clinically relevant research. Medically or scientifically qualified candidates without a PhD or equivalent will be considered, as staff in post are encouraged to study for a higher degree or diploma.

Salary will be at an appropriate point on the Lecturer scale AT2A £12,086-£16,755 per annum. USS Superannuation.

Further particulars may be obtained from the **Personnel Office, University of Edinburgh, 63 South Bridge, Edinburgh EH1 1LS,** with whom applications (8 copies) including curriculum vitae and the names and addresses of two referees, should be lodged not later than August 8th 1990. Please quote reference number 1537. (4070)A

TRANSVAAL MUSEUM
DIRECTOR

Applications are invited for the post of Director of the Transvaal Museum in view of the impending retirement from management of Dr. Bob Brain.

The Transvaal Museum is a state-subsidised natural history museum of international repute with a strong research tradition in the fields of Mammals, Birds, Lower Vertebrates and Invertebrates, Lepidoptera, Coleoptera, General Entomology, Palaeontology and Archaeozoology. It has an excellent natural history Library, Display and Technical Departments, and a staff of 94.

The Director is the Executive Officer of the Board and is expected to provide creative leadership in curation, research and education. A generous salary and service benefits are provided, details of which are available on application. Requirements are a higher degree in natural science and extensive experience in the work of natural history museums, and proficiency in both official languages.

Applications close on August 15th 1990 for appointment on 1st June 1991 and should be addressed to the **Director, Transvaal Museum, P.O. Box 413, Pretoria.** Tel: (012) 3227632, Fax: (012) 3227939. (W7507)A

THE UNIVERSITY OF LEEDS
Department of Biochemistry and Molecular Biology
CHAIR OF BIOCHEMISTRY

Applications are invited for the established Chair of Biochemistry, which will become available on the retirement of Professor Donald S. Robinson in September 1990. This prestigious Chair will be held in a major new department, formed by the merger of the present separate Departments of Biochemistry and Biophysics. Applicants who have an international scientific reputation in any area of biochemistry or molecular biology will be considered. The salary will be within the non-clinical professorial range.

Further particulars may be obtained from the **Registrar, The University, Leeds LS2 9JT** (tel 0532-333969) quoting reference number 83/102. Applications (2 copies) stating age, giving details of qualifications and experience, naming three referees and providing a statement on how, if successful, the applicant would envisage fulfulling the requirements of the post should reach the Registrar not later than 14 September 1990. Applicants from overseas may apply in the first instance by telex (556473 UNILDS G) or facsimile (0532-336017 or 0532-334123), naming three referees, preferably at least one in the United Kingdom.

The University of Leeds is an equal opportunities employer

(4001)A

TECHNICAL DIRECTOR
up to £50,000

Our client is one of Europe's leading biotechnology companies located in the south-east of England. The company is planning a rapid phase of expansion and, as part of the new reorganisation, is seeking a high calibre scientist and manager to direct all research and technical operations.

The successful candidate will have a first class record in the management of biotechnology disciplines including microbiology, medical chemistry and emzymology. Experience in the organisation and running of a wide range of technical projects (budgets of £2-3 millions per annum) is essential and the candidate must possess excellent man management and communicative skills. There will be a seat on the Board and you will report to the Chief Executive.

This is a unique and challenging position, offering a superb opportunity with a rapidly expanding and successful company.

Please send full career details, listing any companies to whom your application should not be sent, to:

Peter McHugh
Cambridge Search
Broadway House
149/151 St Neots Road
Hardwick
Cambridge CB3 7QJ

CAMBRIDGE search RECRUITMENT

(4087)A

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nescot
North East Surrey College of Technology

FACULTY OF SCIENCE & TECHNOLOGY

SCHOOL OF APPLIED PHYSIOLOGY

Senior Lecturer in Physiological Measurement/ Medical Physiology

and

Lecturer in Applied Physiology

Applications are invited from suitably qualified persons to join an established and enthusiastic team concerned with the education and training of students across a range of subjects in Physiology on BTEC, Honours Degree and Professional courses. In addition to suitable academic and professional/research qualifications, relevant hospital, industrial or other experience would be a distinct advantage.

For the Senior Lecturer post, the ability to undertake administrative duties related to course and subject development are essential. Research and consultancy are encouraged.

Salary scale:

SENIOR LECTURER:	£16752 – £20217 p.a. plus £384 London Fringe Allowance
LECTURER:	£9243 – £16752 p.a. plus £384 London Fringe Allowance
	Generous relocation expenses in approved cases

Further information about the above posts and application forms can be obtained from the **Vice Principal, NESCOT, Reigate Road, Ewell, Epsom, Surrey KT17 3DS.** Please send a large stamped addressed envelope. (4065)A

SURREY COUNTY COUNCIL

UNIVERSITY OF KUWAIT HEALTH SCIENCE CENTRE

Faculty of Allied Health Sciences & Nursing

Teaching Appointments in Medical Laboratory Technology

Kuwait University seeks motivated individuals who are attracted by the challenge of preparing young professionals to lead one of the world's fastest developing health care systems into the 21st century.

The Faculty of Allied Health Sciences and Nursing offers 4-year University-based B.Sc. programmes, with practical training carried out in local hospitals. Appointees to all posts will participate in both theoretical and practical teaching, and development and management of their respective programmes. The language of instruction is English.

Positions

a) **Chairman:** At the level of Professor with expertise in any of the disciplines taught within the department and strong administrative experience required.

b) **Haematologist:** At the level of Assistant or Associate Professor — clinical laboratory experienced required.

c) **Clinical Chemist:** At the level of Assistant or Associate Professor.

d) **Histologist:** At the level of Assistant or Associate Professor — experience in both microscopic anatomy and histotechnology required.

Requirements for appointment

1. Professors, Associate Professors and Assistant Professors

   All applicants must possess a Ph.D., or equivalent qualification, in their speciality. Professors must have 14 years experience since their B.Sc., including 8 years since their Ph.D., and have served at least 4 years as an Associate Professor. Associate Professors must have at least 9 years experience since their B.Sc., including 4 years since their Ph.D., and have served at least 4 years as an Assistant Professor. Candidates for Professor and Associate Professor should have a substantial record of research publications in journals of international repute.

Benefits

1. Totals monthly salary within the following scales:

Professor	KD 1070-1230	(8 annual increments)
Associate Professor	KD 875-1035	(8 annual increments)
Assistant Professor	KD 680-840	(8 annual increments)

   1KD = US $ 3.3, UK £ 2.0 approx.

   In addition, for teaching staff who have an active part in the Ministry of Public Health programme, there is a monthly supplement (Lecturer, KD.100; Senior Lecturer, KD.125; Assistant Professor, KD.200; Associate Professor, KD.250; Professor, KD.300) for 10 months a year paid by the Ministry. There is no income tax in Kuwait, and currency is transferable without restriction.

2. An attractive package of associated benefits includes free furnished accommodation, 60 days paid summer leave and 10 days mid-year break for teaching staff, round-trip air tickets, end-of-service gratuity, free medical care in Kuwait government hospital. For Professors, Associate Professors and Assistant Professors furnished accommodation will be provided with water and electricity against deduction of fixed sum from the Social Allowance.

3. Professors, Associate and Assistant Professors also receive a generous baggage and freight allowance, and education allowances for up to 3 children, and may attend one approved conference per year.

Applications

Applications in duplicate, including full curriculum vitae, personal details, comprehensive publication list, 2 recent passport photographs and the names and addresses of 3 referees, should be sent to:

The Dean
Faculty of Allied Health Sciences and Nursing,
Kuwait University Health Science Centre,
P.O. Box 31470,
90805 Sulaibikhat,
Kuwait.

To arrive no later than 30th November, 1990.

Applications for appointments starting later than September 1991 will be considered at anytime.

APPLICATIONS AND CONDITIONS OF SERVICE MAY BE OBTAINED FROM:

EMBASSY OF THE STATE OF KUWAIT
CULTURAL AFFAIRS OFFICE
46, QUEEN'S GATE
LONDON, SW7 5HR

(W7512)A

UNIVERSITY OF EDINBURGH

DEPARTMENT OF BIOCHEMISTRY

Applications are invited for the following post:

LECTURER (temporary)

The post is tenable from 1st January 1991 (or as soon as possible from thereafter) to 31st December 1993. Candidates should be active in any aspect of biochemistry, cell biology or molecular biology. The Department of Biochemistry has a rapidly growing research profile and has teaching responsibilities in the Faculties of Medicine and Science. Strong encouragement will be given to an appointee wishing to develop a research programme either independently, or in collaboration with existing groups, and to attract external funding.

Those wishing to make informal enquiries are invited to telephone the Acting Head of Deparment, Dr David Apps (031-667 1011 ext 2336), and further particulars of the appointment may be obtained from the Personnel Office, 63 South Bridge, Edinburgh EH1 1LS.

Salary will be on the Lecturer scale AT2A (currently £12,086-£16,755 per annum). USS Superannuation.

Applications (5 copies) should include the names and addresses of two referees, and should be lodged with the **Personnel Office, University of Edinburgh, 63 South Bridge, Edinburgh, 63 South Bridge, Edinburgh EH1 1LS** not later than August 15th 1990. *Please quote reference no. 1536.*

(4064)A

RE-ADVERTISEMENT

ROYAL FREE HOSPITAL SCHOOL OF MEDICINE

(University of London)

DEPARTMENT OF IMMUNOLOGY

Research Officer

Research Officer required for project to investigate minimal disease in acute leukaemia using polymerase chain reaction technology. Previous experience with recombinant DNA technology, including PCR, is required. In particular, applicants who have been working with T cell receptor genes and have a broad knowledge of molecular biology and an understanding of lymphocyte development are invited to apply. The position is for three years supported by Leukaemia Research Fund on Research Scale 1A plus £1,767 London Allowance.

Job description available from the **School Office, RFHSM, Rowland Hill Street, London NW3 2PF (tel: 071-794 0500 ext 4262)** to which applications (2 copies of curriculum vitae plus names and addresses of two referees) should be sent by 20 July 1990. Please quote ref: IMM/RO/114.

(4057)A

POSTDOCTORAL POSITION

In virology available immediately to study the molecular biology of RNA-RNA recombination in plant mRNA viruses in NIH-funded project. Experience with molecular techniques desirable. Two/three years available. Salary commensurate with experience.

Send curriculum vitae and three references to: **Dr. J. J. Bujarski, Northern Illinois University, Department of Biology/PMBC, DeKalb, IL 60115. Telephone: 815-753-0601.**

(NW5511)A

ASSOCIATE RESEARCH SCIENTIST

Non-tenure track position in cell physiology and biochemical studies of endothelial biology. Experience required in hybridoma antibody production, protein chemistry and cell culture. Applicant should have experience in studies of endothelial function and a doctoral degree.

Applications with full CV should be sent to **Dr. David Stern, Department of Physiology and Cellular Biophysics, College of Physicians and Surgeons, Columbia University, 630 West 168th Street, New York, NY 10032.** *Columbia University is an Affirmative Action/Equal Opportunity Employer.*

(NW5509)A

POST-DOCTORAL AND PRE-DOCTORAL RESEARCH ASSISTANTS

National Institute for Medical Research and National Heart & Lung Institute

Applications are invited for Postdoctoral and Pre-doctoral positions to work with Drs. F. Anthony Lai and Alan Williams on a 3 year, British Heart Foundation funded project designed to study the structure-function relationships of the calcium release channel (ryanodine receptor) of cardiac muscle sarcoplasmic reticulum.

The Post-doctoral assistant will be based with Dr. Lai at the National Institute for Medical Research, Mill Hill. Experience in molecular biology and biochemical techniques would be an advantage. Ref No: BHF/111.

The Pre-doctoral assistant will be based with Dr. Williams at the National Heart & Lung Institute in central London and will undertake the functional aspects of the project. Experience or interests in single channel techniques or membrane biochemistry would be an advantage and a suitably qualified person would be encouraged to register for a higher degree. Ref No BNF/112.

Applications for both posts should be sent by 20th July 1990, to **Amanda Wilson, Personnel Manager, National Heart & Lung Institute, Dovehouse St., London, SW3 6LY. Tel 071-351 8176. FAX: 071-376 3442.**

Further Information on the project may be obtained from Dr. Alan Williams on 071-352 81221 ext. 3308. (4090)A

THE ERNA AND JAKOB MICHAEL VISITING PROFESSORSHIPS 1991/92

THE JOSEPH MEYERHOFF VISITING PROFESSORSHIPS 1991/92

THE ROSIE AND MAX VARON VISITING PROFESSORSHIPS 1991/92

The Weizmann Institute of Science, Rehovot, Israel offers a limited number of Visiting Professorships in the Natural and Exact Sciences.

The Visiting Professorships are intended for candidates already holding the rank of Full Professor or equivalent and will be for a duration of two months to one year.

Deadline for submittance of completed applications is: November 30, 1990.

For further information or application forms, please contact: **The Academic Secretary, The Weizmann Institute of Science, Rehovot 76100, ISRAEL. (Telex — 972-8-381300, facsimile — 972-8-471667, Bitnet—RSMARJOR@WEIZMANN).** (W7513)A

UCB — PHARMA RESEARCH CENTRE
Brussels, Belgium

CLINICAL NEURO-SCIENTIST

UCB-Pharma is a pharmaceutical company within the UCB Group engaged in long-term research with the aim of developing effective agents for the treatment of disorders of the CNS, the Cardiovascular and the Immune System.

Our Research Centre, located near Brussels, Belgium, is looking for a highly skilled, motivated and creative clinical scientist to join its CNS-Therapeutic research and development unit. The key issue will be the integration of animal models of CNS diseases and clinical validation of such models to discover and develop new treatments of CNS disorders.

The person appointed will be a neuropsychiatrist, neurologist or equivalent with both clinical experience and a laboratory background. He or she will be able to demonstrate productivity in the CNS area by means of publications, etc.

The person appointed will be based at UCB's modern research campus in Braine-l'Alleud, just south of Brussels, so a knowledge of French would be useful, though not essential. Personal qualities should include scientific drive and rigour, flexibility, a creative outlook, and the tact and diplomacy to deal with people at all levels.

This opportunity to develop a whole new range of research processes is of course a senior appointment, and offers excellent remuneration and benefits, together with pleasant working surroundings and generous relocation expenses.

If you feel you can meet this challenge and would like more information, please contact, in the first instance, **Dr Colin WILSHER, UCB R&D UNIT, C/O UCB (PHARMA) Ltd., Star House, 69 Clarendon Road, Watford, Herts WD1 1DJ** — Telephone: 0923 248011. (W7518)A

The **European Southern Observatory (ESO)** is an intergovernmental organization responsible for astronomical research in the southern hemisphere. The eight member states of the ESO are Belgium, Denmark, France, Italy, the Netherlands, the Federal Republic of Germany, Sweden and Switzerland.

The ESO occupies a leading position in the world scientific community as the first European astronomical centre. Its research is of use for a wide range of sciences as well as industry.

EUROPEAN SOUTHERN OBSERVATORY

is looking for an (m/f)

ASTRONOMER/DATA ARCHIVIST (ref. ESD307)

to start work as soon as possible in the Image Processing Group of the Science Division at the ESO Headquarters in Garching near Munich, Federal Republic of Germany.

Assignment: This position will entail maintenance and further development of an archiving system mainly for data acquired with the ESO telescopes in Chile. This includes maintenance and improvement of methods for storing original data, for searching and retrieving these data, and managing the access of users to the archive, general management of data bases relating to the Science Division using a Sybase Date Base Managing System on UNIX based computers as well as development and maintenance of interfaces between such systems and the MIDAS image processing system.

Experience and knowledge: University degree in astronomy, physics, mathematics, computer science or equivalent. Familiarity with data base management systems, computer operations and high level programming languages like C is required, as is experience in the development of science application software. A good background in astronomy and experience in the handling of astronomical data are desirable. Working experience with UNIX would be an advantage. A good working knowledge of English is indispensable: some knowledge of French or German would be an advantage.

Remuneration: The remuneration for this post is commensurate with the background, experience and family status of the candidate. The basic monthly salary (tax-free) will be between DM 4,994 and DM 7,413 to which may be added a non-resident allowance of either 9% (single) or 12% (head of family) as well as some other allowances.

Although preference will be given to nationals of the ESO member states, no nationality is a priori excluded.

Applications, indicating the reference number, should be submitted before August 15, 1990 to

Personnel Administration and
General Services,
European Southern Observatory,
Karl-Schwarzschild-Straße 2,
D-8046 GARCHING b. München,
Federal Republic of Germany
Tel.: (89) 320.06.216-9 (W7515)A

Database Support Programmer

Research Computer Unit

The Imperial Cancer Research Fund is one of the largest charity research institutions in the UK with wide ranging interests in fundamental, applied and clinical research.

Applications are invited for the post of database support programmer to work on the development of a clinical trials database for use in the Fund's Medical Oncology units. Experience of ORACLE or other 4GL programming and some knowledge of 3GL programming methods would be an advantage. The post would also involve some user support and training.

£16,200-£20,000
including pension supplement

Application should be made by sending your curriculum vitae, including names and addresses of 2 referees to the Recruitment Officer, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX. Tel 071-242 0200 ext. 3357, quoting reference 152/R.

SMOKING IS ACTIVELY DISCOURAGED.

Imperial Cancer
Research
Fund

(4103)A

(Woolwich Campus)

Electron Microscope Technician

Required with experience in TEM and its application to biological systems in the School of Materials Science and Physics. You will be expected to prepare biological specimens for TEM use, provide assistance to undergraduate and postgraduate students in the use of the microscope and associated techniques. Duties will also include the general running and maintenance of the microscope. Experience of SEM would be an advantage as would knowledge of light microscopes and their maintenance.

Salary: £13,209 - £14,856 per annum (inclusive).

Further particulars and application form from the Staffing Officer, Thames Polytechnic, Wellington Street, Woolwich, London SE18 6PF, to be returned by 17 July 1990.

THAMES Polytechnic
L·O·N·D·O·N
1890 -1990 Centenary Year
'Seeking to achieve equality of opportunity'

(4092)A

RESEARCH ASSOCIATE

We are seeking an individual with research experience in mechanisms of human airway inflammation. The position provides a unique opportunity for a creative scientist to work independently and in collaboration with a multidisciplinary team of University of North Carolina and U.S. Environmental Protection Agency scientists active in the area of pulmonary cell and molecular biology. The successful candidate should have at least 2 years of postdoctoral training in biological and biochemical approaches to study mediators including neurogenic substances and cytokines involved in the inflammatory/allergic response. Competitive salary and fringe benefits commensurate with education and experience. Interested applicants should send curriculum vitae, a statement of research interests and goals, and the names of three references to: **Dr. Hillel Koren, Center for Environmental Medicine and Lung Biology, University of North Carolina School of Medicine, CB #7030, Medical Research Building C, Chapel Hill, NC 27599.** *Affirmative Action/Equal Opportunity Employer.* (NW5510)A

UNIVERSITY OF CAMBRIDGE
SCHOOL OF CLINICAL MEDICINE
DEPARTMENT OF MEDICINE (NEUROLOGY)

POST-DOCTORAL RESEARCH ASSISTANT

Applications are invited for the position of Post-Doctoral Research Assistant to work on calcium imaging and membrane physiology of glia. The work will be carried out jointly in the Department of Medicine (Neurology) on the Addenbrooke's Hospital Site with Alastair Compston and the Neurosciences Research Group of Smith Kline Beecham in Harlow with Chris Benham. Technical experience in single cell electrophysiology and/or cell culture would be an advantage.

The post is funded by Smith Kline Beecham and is available from 1 July 1990 for three years. The University of Cambridge conditions of service will apply and the salary is in the range £10,458-£15,372 (under review) depending on age and experience.

Applications including curriculum vitae and the names of at least two referees should be submitted to Mr. K. G. Smith by 31 July 1990. Further details may be obtained from **Professor Alastair Compston, University of Cambridge School of Clinical Medicine, Addenbrooke's Hospital, Cambridge CB2 2SP (tel: 0223-217091).**

The University follows an equal opportunities policy. (4074)A

UNIVERSITY OF ABERDEEN
SCHOOL OF BIOMEDICAL SCIENCES

RESEARCH ASSISTANT

Applications are invited for the post of Research Assistant to work on a project concerned with cell lineage in developing mouse muscle. This will involve the use of a replication deficient retrovirus to introduce a marker gene into cells. This will enable the identification and subsequent fate of cell populations to be followed.

Applications should either have a Ph.D. or an Honours Degree in a relevant subject. A knowledge of histology and electron microscopy would be advantageous, but is not essential as training will be given. The post is tenable for one year commencing September 1990. Salary placement on the Scale £10,458-£12,381 per annum depending on qualifications and experience.

Informal enquiries to Dr. Peter Wigmore (0224 273095). Further particulars from the Personnel Office (0224 273500). Applications by letter and CV (two copies) including the names of three referees to the **Personnel Office, University of Aberdeen, Regent Walk, Aberdeen AB9 1FX** by 27th July 1990 quoting reference LG/029. (4109)A

AGRICULTURAL AND FOOD RESEARCH COUNCIL

INSTITUTE OF ANIMAL PHYSIOLOGY AND GENETICS RESEARCH

Babraham, Cambridge CB2 4AT

POSTDOCTORAL POSITION

Signal Transduction Mechanisms in Leukaemia Cells

Following the establishment of a new laboratory in the Department of Immunology by Dr. Denis Alexander, a postdoctoral position funded by the Leukaemia Research Fund is available for research on mechanisms involved in transducing activation signals from cell-surface receptors in human leukaemia cells. The research will have an emphasis on the role of kinases and phosphates (reviewed in Immunology Today 10, 200-205, 1989) and would suit someone with a background in cell biology/enzymology/immunochemistry. The position is available as from 1st August, but candidates wishing to start at any time up to 1st November 1990 will be considered. The position is funded for 3 years in the first instance and will be made at an HSO or SSO level (salary range £10,678-£15,504) depending on qualifications and experience, with a non-contributory superannuation scheme and 25 days leave.

The Institute is situated in pleasant countryside just outside Cambridge and provides an excellent environment for academic research.

Applications containing a full curriculum vitae and the names and addresses of three referees should be sent to the Personnel Office, AFRC Institute of Animal Physiology and Genetics Research, Babraham, Cambridge CB2 4AT, as soon as possible, quoting Ref. DA/ST. Informal enquiries by telephone to Dr. Denis Alexander (Tel. 0223 832312 Ext. 554). (4076)A

UNIVERSITY
OF DUBLIN
TRINITY COLLEGE

INNOVATIONS OFFICER

ENVIRONMENTAL RESEARCH/CONSULTANCY

Applications are invited for th above post at Trinity College Dublin. The person appointe will work with the Director o Innovation Services and wil have special responsibility fo co-ordinating and facilitatin College personnel involved i environmental research an consultancy. The successful candidate will also be responsibl for researching and securin support and funding from E and other sources.

Salary Scale: IR£22,823-IR£29,47

The appointment will be tenabl for a period of three years. A secondment arrangement ma be considered practicable.

Further particulars and application forms may be obtained from:

Staff Office,
Trinity College,
Dublin 2.
Telephone: 772941 Ext. 1678/
FAX 772169

The closing date for receipt of completed applications will be *20th July, 1990.*

Trinity College is an Equal Opportunities Employer.

(4091)A

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nature
Still first with the best in science

UNIVERSITY OF BRISTOL

Departments of Microbiology & Pathology

Research Assistant

pplications are invited for the post of post-doctoral Research ssistant funded by a project grant from the Wellcome Trust, hich as 2½ years to run and is to investigate herpes simplex rus-induced demyelinating disease.

he successful applicant will join a multi-disciplinary programme hich is well-funded in this field. Experience in molecular ology, immunology, histology .or cell-culture techniques dvantageous although training available in all of these areas.

alary within the range £10,458-£16,665 (Research 1A under revi- on from 1.5.90) according to age, qualifications and experience.

urther information may be obtained from Dr S Love (0272 701212 xt. 2382), Dr T Hill (0272 303030 ext. 4908) or Dr N Maitland (0272 03454).

or further details telephone 303136 (ansaphone after 5 p.m.) or rite to the **Personnel Office, University of Bristol, Senate House, ristol BS8 1TH**, quoting reference number 294.

n Equal Opportunities Employer. (4062)A

LIVERPOOL HEALTH AUTHORITY

Department of Chemical Pathology

INICAL BIOCHEMISTS — 2 POSTS

1) Grade B salary £15,999 – £17,304
2) Grade B salary £12,644 – £13,676

quired to work in the above artment recently affiliated to University of Liverpool.

he department is part of a jor teaching hospital, covers a nprehensive analytical service has its own 4-bedded meta- ic unit and associated clinics.

ou will be expected to have vious clinical laboratory erience and to contribute to utine and development work he department. Post (1) development of interests associated with nutrition and metabolism, cell culture and bio assay or molecular biology associated procedures; post (2) in the trace and toxic metal section.

Qualifications required are a first or second class honours degree in biochemistry or related subject and preferably a higher degree PhD and/or MCB/ MRCPath. Opportunities will be available to study for a professional qualification if required.

Further information is available from Professor A. Shenkin or Dr. N.B. Roberts, telephone number 051-709 0141 ext 2823/1.

Application forms can be obtained from the Personnel Department, Royal Liverpool Hospital, Prescot Street, Liverpool, tel no. 051-709 0141 ext 2253. Closing date 19th July 1990.

(4072)A

ROYAL POSTGRADUATE MEDICAL SCHOOL

(University of London)

DIVISION OF MOLECULAR ENDOCRINOLOGY & METABOLISM

GRADUATE RESEARCH ASSISTANT

Applications are invited from graduates to join a dynamic team working in the rapidly expanding field of cell regulatory mechanisms in both physiological states. The current programme centres on the hypothalamic pituitary axis and the islets of Langerhans. We have a policy of encouraging all suitable candidates to register for a PhD.

Techniques used in the Unit include radioimmunoassay, receptor binding assays, reverse haemolytic plaque assays, cell culture, nortern blotting and fluorescence activated cell sorting. Experience with any of these techniques would be helpful but we expect to provide full training as required. Applicants should have an interest ir. biology and a reasonable class degree, preferably in a biological subject.

Salary will be in the range £7,594 – £10,903 plus £1,580 London Allowance. Please send full CV with names and telephone numbers of two referees to the **Personnel Office, Royal Postgraduate Medical School, Du Cane Road, London W12 0NN** quoting ref: AMV1. Informal visits to the laboratories can be arranged by telephoning Dr M A Ghatei on 081-740 3044.

Closing Date: 19 July 1990. (4067)A

MEDICAL RESEARCH COUNCIL

COLLABORATIVE CENTRE

The MRC Collaborative Centre was founded in 1986 to act as a link between MRC basic research units and industry. At the Collaborative Centre, commercially-orientated research projects are carried out with scientific support from within the MRC and financial support from industry. Located adjacent to the National Institute for Medical Research in the pleasant semi-rural surroundings of Mill Hill, the Centre has been recently refurbished and is equipped to a high standard. In addition, staff at the Centre have access to a range of computing, library, technical, and recreational facilities at the NIMR.

The **Antibody Engineering Group** at the Collaborative Centre carries out projects focusing on the cloning and manipulation of immunoglobulin genes to create novel diagnostic and therapeutic agents. We currently have two new openings. Appointments will be for two years in the first instance with the possibility for extension.

SCIENTIFIC OFFICER

We are seeking a scientist with experience in recombinant DNA technology to work on the cloning and expression of immunoglobulin genes. The salary will be on the Civil Service Scientific Officer scale ranging from £10,646 to £13,738 per annum (inclusive of London Weighting). An optional MRC pension scheme is available with a 4.5% salary supplement. Applicants should possess a first degree, BTEC, or equivalent qualification. Please telephone for an application form, quoting reference number CC/AE SO2, on 081 959-3666 x 2270, or write to **Ms. Frances Griffiths, Personnel Officer, National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA.**

POSTDOCTORAL SCIENTIST

We are seeking a molecular biologist with practical experience in recombinant DNA technology and with an interest, and preferably some experience, in protein engineering and/or molecular immunology. The salary will be in the range £12,225-£22,229 per annum (inclusive of London Weighting). An optional MRC pension is available. Applicants should send a curriculum vitae and the names of two referees to **Mr. C. R. Russell, Administrative Manager** at the above address, quoting reference number CC/AE PD2.

Informal enquiries for either of the positions may be made to Dr. M. M. Bendig on 081 906-3811. Applications are requested as soon as possible.

The MRC is an Equal Opportunity Employer. (4113)A

STUDENTSHIPS

UNIVERSITY OF ABERDEEN

SCHOOL OF BIOMEDICAL SCIENCES

3 YEAR ANATOMICAL SOCIETY STUDENTSHIP

Applications are invited for a 3 year studentship to work on cell lineage in developing mouse muscle. This will involve the use of a replication deficient retrovirus to introduce a marker gene into cells. This will enable identification and subsequent fate of cell populations to be followed.

It would be an advantage for candidates to have a knowledge of histology and electron microscopy although training will be given. Applicants should have at least an Upper Second Class Degree in a relevant subject and must be British or Irish subjects.

Applications including a full CV and the names of two referees, or informal enquiries, should be made to Dr Peter Wigmore, School of Biomedical Sciences, Division of Anatomy, Marischal College, Aberdeen AB9 1AS (tel. 0224 273095). (4111)F

Research Studentship

A Studentship is available for work on *cloning, structural determination and localisation of neurotransmitter and neuropeptide receptors in a variety of species.* Further information is available from Dr Karin Eidne (tel: 031 229 2575).

Applications, including the names of two referees, to **Ena McKay, MRC Reproductive Biology Unit, Centre for Reproductive Biology, 37 Chalmers Street, Edinburgh, EH3 9EW.** (4112)F

DEPARTMENTS OF BIOCHEMISTRY AND MEDICINE
MUSCLE RESEARCH CENTRE

POSTGRADUATE STUDENTSHIP

Supported by the Muscular Dystrophy Group of Great Britain and Northern Ireland and leading to the degree of Ph.D. The project, which carries a stipend of £6,000 p.a. + fees + travel expenses, will examine the genomic lesions and genetics of McArdle's disease, a heritable deficiency in muscle glycogen phosphorylase that leads to severe disruption of muscle energy supply. The student will be based in the Department of Biochemistry (Dr R. J. Beynon) but will also work closely with Professor R. H. T. Edwards in the Department of Medicine.

For further details contact Dr Beynon on 051-794 4359 by whom applications should be received as soon as possible at the address below.

DEPARTMENT OF GENETICS AND MICROBIOLOGY
SCHOOL OF LIFE SCIENCES

NERC-CASE PH.D. STUDENTSHIP

Tenable for three years from 1 October, 1990.

To develop molecular methods for monitoring survival and gene transfer in recombinant pseudomonads released into aquatic environments. The project will be supervised jointly by Dr J. R. Saunders (Liverpool) and Dr R. W. Pickup of the Institute of Freshwater Ecology, Windermere, Cumbria where the student will spend 3-6 months.

Applicants should have or expect to obtain a first or second class honours degree and have some knowledge of molecular genetic and/or immunological methods.

Enquiries and applications together with the names and addresses of two referees should be received as soon as possible by Dr J. R. Saunders, Department of Genetics and Microbiology, at the address below. (Tel: 051-794 4416).

PH.D. STUDENTSHIPS

Available for the following projects:

Heavy metal resistance genes in bacteria from polluted soil. Professor D. A. Ritchie, Dr P. Strike and Professor S. T. Williams (3624).

NERC/CASE Award with Institute of Freshwater Ecology, Windermere, on Molecular approaches to monitoring the fate and activity of recombinant pseudomonads in soil and aquatic sediments. Dr J. R. Saunders and Dr R. W. Pickup (4416).

Applications are invited from students who either hold, or expect to obtain, an Upper Second class Honours degree in a relevant subject.

Further information can be obtained by ringing 051-794 plus the appropriate number in brackets, or in case of difficulty, the Secretary on 051-794 3622.

Applications, together with a cv and the names and addresses of two academic referees, with the title of the preferred project(s) should be addressed to the named supervisor, Department of Genetics and Microbiology, at the address below.

The University, PO Box 147, Liverpool L69 3BX

University of LIVERPOOL

AN EQUAL OPPORTUNITY EMPLOYER (4094)F

THE UNIVERSITY OF BIRMINGHAM/
ICI PHARMACEUTICALS

MRC COLLABORATIVE PH.D STUDENTSHIP

THE WHITE CELL RESPONSE IN ISCHAEMIA

Department of Haematology, The Medical School

A postgraduate studentship is available, based in the Medical School, but with significant time spent in the ICI Pharmaceuticals Bioscience Laboratory, Alderley Park, Macclesfield. The project is a study of changes in the flow behaviour of white cells caused by ischaemia, and possible therapeutic interventions. The work is inter-disciplinary and those with backgrounds in the biological or physical sciences are invited to apply. A supplementation of £1200 to the standard stipend will be paid.

Further information and application forms (returnable by 2 August 1990) from **Dr G Nash, Department of Haematology, The Medical School, Birmingham B15 2TT. Tel: 021 414 4040.** (4097)F

Research Studentships in Biochemistry

DEPARTMENT OF BIOCHEMISTRY
UNIVERSITY OF DUNDEE

A number of CASE (denoted ¶) and earmarked Research Studentships are available for the following projects:

Dr. B Spruce & *Prof. D Glover*	The intracellular trafficking and biological role of a mammalian neuropeptide procursor within neuro-endocrine and glial cells ¶
Dr. D Boxer	Regulation and biosynthesis of metalloenzymes in *E. co* Structural analysis of nickel-binding proteins in *E. coli* ¶
Dr. S W Homans	Three-dimensional structure of biologically active oligosaccharides by NMR and computational methods
Prof. D M J Lilley	Nucleic acid structure and DNA-protein interactions
Dr. M J R Stark	Development of molecular genetics in the yeast *Pichia pastoris* ¶

These places will all be supplemented in the first year with a bursary of £250 (£500 for candidates with a First Class Honours degree). The Department is one of the most successful and highly rated in the UK for biochemistry research and is very well equipped and funded. In addition to the outstanding research facilities and working environment, Dundee boasts excellent access to outdoor recreational facilities and accommodation/living expenses are among the lowest in Britain.

Applications, including a full curriculum vitae and the names and addresses of two referees should be sent immediately to **Dr M J R Stark, Department of Biochemistry, The University, Dundee DD1 4HN (tel. 0382-23181 ext. 4250)**. Prospective applicants are strongly encouraged to phone first. Candidates should have (or expect to have) at least an upper second class honours degree and be normally resident in the U.K.

(4105)F

ROYAL FREE HOSPITAL SCHOOL OF MEDICINE
(University of London)
DEPARTMENT OF PHARMACOLOGY

CASE STUDENTSHIP IN COLLABORATION WITH ICI PHARMACEUTICALS

FOR A PROJECT ON "ENDOTHELIUM-DERIVED VASOACTIVE FACTORS, LIPOPROTEINS AND ATHEROSCLEROSIS

Applications are invited for a CASE studentship under the supervision of Dr M. Jacobs, to study the role of lipoproteins in the alteration of vascular responses by atherosclerosis. Applicants should hold a first or upper second class honours degree in Pharmacology or related subjects and wish to undertake research for a PhD in Pharmacology.

The studentship is tenable for 3 years starting October 1990. Informal enquiries should be made to **Dr M. Jacobs, Department of Pharmacology, RFHSM, Rowland Hill Street, London NW3 2PF (tel: 071-794-0500 ext 4300)** to whom applications, including curriculum vitae and the names and addresses of two referees, should be sent. (4068)F

ROYAL FREE HOSPITAL SCHOOL OF MEDICINE
(University of London)
DEPARTMENT OF BIOCHEMISTRY

SERC PhD STUDENTSHIP

Applications are invited for a studentship to work with Dr S. J. Perkins (Biochemistry) and Dr V. C. Emery and Dr J. E. Grundy (Virology) for biochemical and structural studies of viral proteins in cytomegalovirus and papillomavirus using synthetic peptides and/or recombinant DNA techniques and sequence analyses. Excellent facilities in new laboratories are available.

Applicants should have a 1 or 2-i in Biochemistry or other relevant subject. The award will be supplemented by £500 p.a., and there will be opportunities for part-time demonstrating.

Applications, including a curriculum vitae and names of two academic referees, should be sent as soon as possible to **Dr V. C. Emery (071-794-0500 ext 3109), Department of Virology, RFHSM, Rowland Hill Street, London NW3 2PF,** from whom further details can be obtained. (4059)F

DEPARTMENT OF MICROBIOLOGY
UNIVERSITY OF SURREY
BIOTECHNOLOGY DIRECTORATE
PhD STUDENTSHIPS
(quota and CASE)

vo studentships are available for three years from October 1990:

Molecular studies on cellular physiology.

e objective is to study the production of ammonia by animal cells in lture. The project will involve the expression of cloned genes in ammalian cells and studies on the effect of this expression on llular physiology, particularly with regard to the production and ilisation of ammonia.

e studentship will provide research training in aspects of molecular ology, cell physiology and the large scale culture of cells in bioreactrs.

r further infomation contact Prof. R.E. Spier (0483 571281 ext. 2662) Dr. P.G. Sanders (0483 571281 ext. 2665).

Factors affecting secondary metabolite production in bioreactors.

e objective is to quantify the influence of physical, physiological d molecular factors on antibiotic yield and gain some insight into e reasons why batch to batch variability exists between successive oreactors runs. This is a CASE studentship with Shell UK Ltd.

esearch training will be provided in fermentation physiology, electon microscopy and biochemical/molecular techniques.

r further information contact Dr. M.E. Bushell (0483 509277).

epartment of Microbiology, University of Surrey, GUILDFORD, urrey, GU2 5XH.

pplications, including CV and the names of two referees should be ent to Dr. P.G. Sanders or Dr. Bushell ASAP but no later than 20th uly 1990. (4098)F

DIVISION OF PHYSIOLOGY
UNIVERSITY OF ABERDEEN

Applications are invited for a University funded Ph.D. studentship in the Division of Physiology starting October 1990. The project will be supervised by Stephen Davies and will involve the use of electro-physiological techniques to study the mechanisms of synaptic transmission and synaptic plasticity in brain slice preparations. The stipend will be at the same level as Research Council studentships and candidates should hold, or expect to hold, an upper second or first class degree. Informal enquiries may be made by telephone on (0224) 273016/273010, or in writing to **Dr. S. N. Davies, Division of Physiology, School of Biomedical Sciences, Marischal College, Aberdeen, AB9 1AS**. Replies as soon as possible please. (4108)F

UNIVERSITY OF LONDON
ROYAL HOLLOWAY AND
BEDFORD NEW COLLEGE

PETROLEUM SCIENCE AND
TECHNOLOGY INSTITUTE
EDINBURGH

PhD Research Studentship on FAULT SEALS

The research objective is to identify common processes which control ault seals and determine the conditioins at which they operate. This will have direct bearing on subsurface fluid migration. The student will undertake extensive field work in the Western Alps and Britain, and examine cores from North Sea wells. A wide range of analytical echniques will be used to investigate deformation mechanisms and chemical processes in fault zones, using XRD, XRF, cathodoluminescence, fluid inclusions, and stable and radiogenic isotopes. The student will also gain experience working in oil company offices examining seismic data, and electric logs.

Petroleum Science and Technology Institute offer a stipend of c. £5,550 per annum and excellent research support.

Please send CV and names of two academic referees to **Dr. Ian Davison, Dept. of Geology, Royal Holloway and Bedford New College, Egham, Surrey, TW20 0EX**. For informal enquiries Tel. 0784 43615. (4095)F

THE UNIVERSITY OF BIRMINGHAM
DEPARTMENT OF OPHTHALMOLOGY
RESEARCH STUDENTSHIP
Birmingham Hospital's Endowment Fund
Infant Visual Development:
"The Design and Use of Acuity Cards"

Applications are invited from graduates in Psychology, Physiology, Optometry or other relevant discipline.

For further details, please contact **Professor Alistair Fielder or Dr Merrick Moseley, Academic Unit, Birmingham and Midland Eye Hospital, Church Street, Birmingham B3 2NS**. Tel: 021 236 4911 Ext 245.

The University is an equal opportunities employer. (4085)F

UNIVERSITY OF CAMBRIDGE
DEPARTMENT OF BOTANY
SERC CASE STUDENTSHIP

in plant biochemistry/molecular biology to work on the isolation, characterization and sequencing of plant inositol phosphate phosphatases. The project is in conjunction with Merck Sharp & Dohme Research Laboratories Neuroscience Research Centre, Harlow.

Applications with CV and names of 2 referees to **Dr D.E. Hanke, Department of Botany, Downing St, Cambridge CB2 3EA** from whom details can be obtained. Informal enquiries can be made on 0223-333913. (4078)F

UNIVERSITY OF BRISTOL
H. H. WILLS PHYSICS LABORATORY
SERC/CASE STUDENTSHIP

Applications are invited for a Ph.D. Studentship to use scanning tunnelling microscopy, x-ray techniques, conventional electron microscopy, and rheological measurements to study the structure and properties of a range of wheat gluten protein subunits. The project, which is funded for three years, is with Dr. M. J. Miles (Physics) and Professor P. R. Shewry of Long Ashton Research Station (University of Bristol), where the student will initially isolate and purify the proteins.

Please apply to **Dr. M. J. Miles, Department of Physics, University of Bristol, Bristol, BS8 1TL**. Applications should be by letter and should include a CV and the names and addresses of two referees. (4063)F

Studentships continued on page 20

ASSISTANTSHIPS

UNIVERSITY OF WARWICK
DEPARTMENT OF BIOLOGICAL SCIENCES
POSTGRADUATE RESEARCH ASSISTANT

A Postgraduate Research Assistantship (IB), funded by the SERC Invertebrate Neurobiology Initiative, is available to work on the analysis of cell lineage in the Drosophila nervous system using the yeast FLP recombination system (see Golic & Lindguist, Cell 59: 499). Interest or experience in molecular biology, genetics or animal development would be an advantage.

Salary will be on the Research IB scale: £9816-£12381 pa (under review). Further particulars available from Dr C J O'Kane (0203 523523 ext 2812/2559). Application forms from the **Executive Officer (0203 523517), Department of Biological Sciences, University of Warwick, Coventry CV4 7AL** quoting Ref No: 48/A/89. Closing date 27 July 1990. (4088)P

Assistantships continued on page 20

STUDENTSHIPS

UNIVERSITY OF READING
School of Plant Sciences

SERC-CASE STUDENTSHIP

Sponsored by the Institute of Horticultural Research, Littlehampton

PLANT MOLECULAR BIOLOGY

A studentship is available in the Department of Botany to work with Dr. C.B. Johnson on the Molecular biology of phytochrome action. The student will spend up to 12 months at Littlehampton, working with Dr. B. Thomas in the Department of Biochemistry and Molecular Biology.

Applicants should hold or expect a first or upper second class honours degree in a relevant subject.

Apply, giving names and addresses of two referees, to **Dr. C.B. Johnson, University of Reading School of Plant Sciences, Whiteknights PO Box 221, Reading RG6 2AS** (Telephone 0734 875123, Ex. 4085) from whom further details can be obtained.

(4071)F

ASSISTANTSHIPS

Coláiste na hOllscoile Corcaigh
University College Cork

UNIVERSITY COLLEGE CORK

RESEARCH ASSISTANTSHIP

Applications are invited for a Research Assistantship on an E.C funded laboratory and field investigation into the biology and prevention of Proliferative Kidney Disease (PKD) in salmonids, from Biological Sciences graduates with an M.Sc or Ph.D

Enquiries and applications to:

Professor Maire Mulcahy,
Department of Zoology,
University College,
Cork, Ireland.

University College Cork is an equal opportunities employer. (4081)P

nature
— the professionals' choice

FELLOWSHIPS

University of Newcastle upon Tyne

Postdoctoral University Fellowship

Departments of Obstetrics/Physiological Sciences

A Research Fellowship, funded by the University of Newcastle upon Tyne, is available for one year to investigate the cellular physiology of human pregnant myometrium. The project involves the measurement of force production and intracellular ion changes by microspectrofluorimetry in isolated tissues. Experience in cellular physiology is requested.

Salary will be within the range £10,458 to £12,381 p.a. on the Grade 1A scale according to qualifications and experience.

Further information can be obtained by writing to, or telephoning **Dr. J.I. Gillespie, Department of Physiological Sciences, The Medical School, Framlington Place, Newcastle upon Tyne NE2 4HH** (Tel. 091-222 7010) with whom applications, with full c.v. and the names and addresses of three referees, should be submitted by 3rd August 1990. (4066)E

University of Cambridge

Postdoctoral Fellowship in Neurobiology

A postdoctoral position will be available from Sept 1st 1990 with Prof M Burrows to study the function of local neurones in controlling the locomotion of an insect. The post, which is funded for 3 years by the Human Frontier Science Program, offers the opportunity to join an active multidisciplinary research group and to participate in collaborations with laboratories in Berlin F.R.G., Hokkaido Japan, and Cal. Tech U.S.A.

Salary typically in the range £11,088-12,879 depending on age and experience.

Please contact, or send applications, including the names of 2 referees to,

Prof. M. Burrows,
Department of Zoology,
Downing Street,
Cambridge CB2 3EJ.
Tel 0223 336628.

(4096)E

ESO FELLOWSHIPS 1991-1992

The European Southern Observatory (ESO) intends to award up to
post-doctoral fellowships tenable in the ESO Headquarters, located
Garching near Munich.

The main areas of activity are:

- to do research in observational and theoretical astrophysics;
- to carry out a program of development of instrumentation for t
  La Silla telescopes;
- to develop future telescopes involving new technology;
- to provide data reduction facilities for users of the ESO instr
  ments;
- to provide photographic facilities for atlases of the southern sky
- to foster cooperation in astronomy and astrophysics in Europe.

Fellows normally participate in one or more of the above. In additic
there is the possibility of participating in the activities of the Europea
Coordinating Facility of the Space Telescope (ST-ECF) which h
been established at ESO.

Fellows will normally be required to spend up to 25% of their time
supporting activities such as the introduction of users to data reductic
facilities, remote control operations and testing new instrumentation.

Fellowships are to be taken up between January and October 1991.

Most of the scientists in the Centre come from the Member States
ESO, but several are from other countries. The Member States of ES
are: Belgium, Denmark, the Federal Republic of Germany, Franc
Italy, the Netherlands, Sweden, and Switzerland. In addition to reg
lar staff members, the Centre comprises visiting scientists, pos
doctoral fellows, and graduate students.

ESO facilities include the La Silla Observatory in Chile with its eig
telescopes in the range 0.9 to 3.6m, as well as a 1 m Schmidt, the 15
SEST and smaller instruments. In Garching, extensive measurin
image processing and computing facilities are available.

Applicants normally should have a doctorate awarded in rece
years. The basic monthly salary will be not less than DM 4341 to whic
are added 7% for pension purposes and a non-residence allowance
9-12% if applicable. The fellowships are granted for one year, wit
normally a renewal for a second year and occasionally a third yea
Applications should be submitted to ESO not later than 15 Octobe
1990. Applicants will be notified in December 1990. The ESO Fellow
ship Application form should be used. Three letters of recommendation from persons familiar with the scientific work of the applicant should be sent to ESO directly. These letters should reach ESO not later than 15 October 1990.

Enquiries, requests for application forms and applications should be addressed to:

European Southern Observatory
Fellowship Program
Karl-Schwarzschild-Straße 2
D — 8046 GARCHING b. München
Federal Republic of Germany

(W7514)E

EMBO

EUROPEAN MOLECULAR BIOLOGY ORGANIZATION

SHORT TERM FELLOWSHIPS in molecular biology

The European Molecular Biology Organization awards, to scientists working in Europe and Israel in the field of molecular biology and allied disciplines, short term fellowships of one week up to three months duration. The fellowships are to support collaborative research between laboratories in different countries and provide a travel grant and subsistence allowance. Applications may be made at any time and are decided upon soon after the receipt of application.

Applications for exchanges between laboratories within any one country cannot be considered. Fellowships involving transatlantic travel are awarded only in extremely exceptional circumstances. Inquiries should be accompanied by a self-addressed adhesive label.

Application forms and further details may be obtained from Dr J. Tooze, Executive Secretary, European Molecular Biology Organization, 69 Heidelberg 1, Postfach 1022. 40, F.R.G. (W6896)E

continued on page

FELLOWSHIPS continued

POSTDOCTORAL FELLOWSHIPS, MOLECULAR EVOLUTIONARY GENETICS, PENN STATE

wo Postdoctoral positions are available for work with faculty nembers in the Institute of Molecular Evolutionary Genetics in a ariety of research areas, including theoretical population genetics, he sequencing of genes in bacteria, Drosophila, amphibians, and ther organisms, the statistical analysis of nucleotide sequences, and e epidemiological and biosystematic applications of molecular ata. Applicants should send a curriculum vitae and the names of two eferences to: **DR. ROBERT K. SELANDER, CHAIRMAN, SEARCH OMMITTEE, DEPARTMENT OF BIOLOGY AND INSTITUTE OF IOLECULAR EVOLUTIONARY GENETICS, BOX A3, 208 MUELLER ABORATORY, PENN STATE UNIVERSITY, UNIVERSITY PARK, PA 6802.** APPLICATION DEADLINE: AUGUST 5, 1990. *An Affirmative ction/Equal Opportunity Employer. Women and Minorities Encour-ged to Apply.* (NW5499)E

GRANTS & SCHOLARSHIPS

NATIONAL UNIVERSITY OF SINGAPORE

RESEARCH SCHOLARSHIPS

Research Scholarships are available to outstanding university graduates registered for, or admissible to, master's and doctor's degree programmes by research at the National University of Singapore.

The Scholarships are tenable for one year in the first instance and, subject to satisfactory progress, renewable annually for another two years.

The value of each Scholarship ranges from S$1,000.00 to S$1,500.00 per month (£$1.00 = S$3.11 approximately) and is not subject to income tax; the actual quantum is determined on the candidate's qualifications and experience. No travel allowance is provided.

Research Scholars may be asked to assist in the work of the Departments to which they are attached for not more than six hours a week. They can earn up to S$5,400.00 (gross) per annum at the current rate of remuneration from such departmental work.

The University will assist Research Scholars in obtaining accommodation.

Application forms may be obtained from:

The Registrar
NATIONAL UNIVERSITY
OF SINGAPORE
10 Kent Ridge Crescent
Singapore 0511
Republic of Singapore
Telex: UNISPO RS 33943
Telefax: 7786371

NUS Overseas Office
5 Chesham Street
London SW1, UK

In your request for application forms, please state:-

(a) the field of research you intend to undertake; and
(b) your highest educational qualification.

Applications should be received by the Registrar, National University of Singapore preferably by **31 August 1990.** (W7498)H

AWARDS

MEMORIAL UNIVERSITY OF NEWFOUNDLAND DEPARTMENT OF PHYSICS

NSERC FACULTY AWARDS FOR WOMEN

The Department of Physics at Memorial University of Newfoundland is seeking nominees for the *Women's Faculty Awards* recently instituted by the Natural Sciences and Engineering Research Council of Canada. The terms and conditions of these awards are similar to those for the now discontinued University Research Fellowship (URF) program. It is expected that successful candidates will be appointed at the rank of Assistant Professor as of July 1, 1991. Both experimentalists and theoreticians are encouraged to apply. While the area of Condensed Matter Physics is of special interest, qualified candidates in the areas of Physical Oceanography and Atomic and Molecular Physics will also be given serious consideration. All candidates are required to be Canadian citizens or landed immigrants. Applications should include a curriculum vitae with a list of publications and a selection of representative reprints, a research proposal, and the names of at least three referees. Correspondence should be directed as below with a deadline of September 1, 1990.

Dr. M. J. Clouter, Head
Department of Physics
Memorial University of Newfoundland
St. John's, NF
Canada A1B 3X7
Tel.: (709) 737-8738
Fax: (709) 737-8739

(NW5504)N

COURSES & CONFERENCES

PHARMACIA LKB BIOTECHNOLOGY

"Manage Your Biomolecules"

RESIDENTIAL TRAINING COURSES FOR 1990

- Advanced Molecular Biology Techniques made simple.
  Cambridge, 4 days, £430.00 + VAT
  31st July - 3rd August.
- Protein Chromatography with Electrophoresis.
  Warwick, 3 days, £345.00 + VAT
  14th - 16th August.
- Working with Peptides
  Warwick, 3½ days, £395.00 + VAT
  21st - 24th August.
- Protein Chromatography with Electrophoresis.
  Bath, 3 days, £345.00 + VAT
  18th - 20th September.

For all course information, please contact: Donna Harris at Pharmacia House on (0908) 661101 ext. 3892.

Pharmacia

Advancing The New Biology

(4110)C

British Council International Medical Course

Immunotherapy with monoclonal antibodies

7-13 April 1991, Cambridge

The aim of this course is to demonstrate the way that basic science can lead to the application of monoclonal antibodies in therapy. The programme will consist of lectures and discussions.

Topics to be covered will include: ways of harnessing natural effector mechanisms to permit antibodies to kill target cells; targeting drugs, isotopes and toxins to tumour cells; engineering antibodies using recombinant DNA methods; bispecific antibodies to direct T-cells to tumours; antibodies as immunosuppressants; animal models of transplantation tolerance with monoclonal antibodies; monoclonal antibodies in the treatment of auto-immunity; monoclonal antibodies in bone-marrow transplantation; antibody therapy in lymphoma and leukaemia; avoiding antiglobulin responses.

The Director of Studies will be **Herman Waldmann**, FRS, Kay Kendall Professor of Therapeutic Immunology, University of Cambridge. The Assistant Director will be **Dr Geoffrey Hale**, Senior Research Associate, Cambridge University Department of Pathology.

The course is designed for basic scientists interested in the application of immunological knowledge to therapy; academic rheumathologists; clinical immunologists; academic transplantation physicians/surgeons and tumour immunologists.

There are vacancies for 30 participants.

Course fee: £725; accommodation charge: £200; total fee £925.

The course sessions will take place at Downing College Cambridge where participants will be accommodated in single study-bedrooms. Bathroom facilities will be shared.

Further information and application forms are available from British Council Representatives overseas or from Courses Department, The British Council, 10 Spring Gardens, London SW1A 2BN.

The
British
Council

(4082)C

IBC TECHNICAL SERVICES ARE PLEASED TO ANNOUNCE THE FOLLOWING FORTHCOMING CONFERENCES:

(1) **Bioseparations '90**

New Technologies in Upstream and Downstream Processing
27th/28th September 1990, Cambridge, UK
Chaired by Dr C.R. Lowe and Dr. H.A. Chase

(2) **New Horizons in Dermatology**

Recent Breakthroughs and Likely Developments in the 90's
1st/2nd October 1990, London
Chaired by Professor M Greaves and Professor R Marks

(3) **New Developments in Free Radical Research**

15th/16th October 1990, London
Chaired by Professor T. Slater and Professor H.S. Jacob

(4) **Advances in Inflammation Research**

The Impact of New Developments
31st October/1st November 1990, London

(5) **Biotechnology in the Food Industry**

New Applications and Novel Approaches
26th/27th November 1990

(6) **Monoclonal Antibodies for Clinical Use**

28th/29th November 1990, London

(7) **Potassium Channels '90**

Structure, Modulation and Clinical Exploitation
6th/7th December 1990, London
Chaired by Professor A Weston

For a full conference programme on any of the above events, either telephone Renata Duke in London on 071-236 4080, fax on 071-489 0849 or write to her at **IBC Technical Services, Bath House, 56 Holborn Viaduct, London EC1A 2EX.** (4079)C

IBC

University of Toronto
RECONSIDERING VELIKOVSKY
August 17-19, 1990

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Preservation and transfer of eggs and embryos (D. Whittingham, London; B. L. Dresser, Cincinnati; G. J. Morris, Cambridge)

Monitoring and managing fertility *in vivo* and *in vitro* (J. K. Hodges, Gottingen; H. D. M. Moore, London).

Wednesday 5 September

Genetic challenges to long-term population management (A. Schreiber, Heidelberg; E. A. Oakenfull, Oxford)

The role of DNA technologies (D. T. Parkin, Nottingham; W. Amos, Cambridge; J. Avise, Georgia; O. A. Ryder, San Diego)

ntegrating technologies into breeding programmes (J. Ballou, Washington D.C.; G. M. Mace, London; G. Lucas, Kew Gardens; R. Frankham, Macquarie University, Australia)

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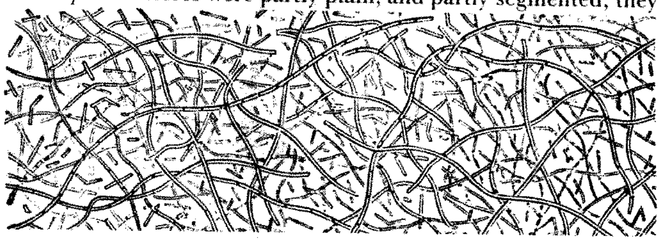
120 YEARS AGO

A FLASK containing a filtered infusion of turnip *in vacuo*, which had been hermetically sealed only *five* days previously, after the fluid had been boiled, was opened on December 15, 1869. On the second day after the flask had been sealed, the previously clear solution began to exhibit a cloudy appearance. The next day a reticulated scum was seen on the surface of the fluid, which gradually became more manifest on the two following days. When the neck of the flask was opened, its contents were found to emit a most fœtid, sickly odour. Microscopical examination revealed a very large number of bacteria and vibrionic-looking rods, some straight and others bent, some motionless and others exhibiting languid movements. These, mixed up with a thickly interlaced network of *Leptothrix* filaments, constituted the reticulated pellicle which was seen on the surface. The *Leptothrix* fibres were partly plain, and partly segmented; they had a precisely similar appearance to the vibrionic-looking filaments, with which in thickness they also closely agreed. The long filaments seemed, in fact, to be only developed forms of the shorter rod-like filaments. A large nucleated ovoid cell was also seen, $^1/_{1428}''$ in its longest diameter, as well as a smaller vesicle, enclosing a rod-like body.


Bacteria, *Vibrios*, and *Leptothrix* filaments, met with in a Turnip-infusion which had been only five days *in vacuo*.

"Facts and reasonings concerning the heterogenous evolution of living things", H. Charlton Bastian.

DEATH has been busy lately among the more eminent members of the medical profession. We have this week to record the decease of Sir James Clark, Bart., M.D., F.R.S., chief physician to Her Majesty, which took place on the 29th ult., in the 82nd year of his age. Sir James had held the appointment of physician to several members of the Royal Family, was the author of several works on climate and on consumption, and was a member of the Senate of the University of London. He attended the poet, John Keats, during his last illness at Rome in 1821.
From *Nature* July 7, 1870.

100 YEARS AGO

ACCORDING to the *Ceylon Observer*, Mr. A. T. W. Marambe, of Kandy, the translator of "Gulliver's Travels" and the author of "A practical Synopsis of Ceylon History," has in preparation a little work on the Veddah language. Many have attempted this task before, but without success. Besides Veddah songs, a description of habits and customs, &c., the book will have a completer list of words than has hitherto appeared.

THE Norwegian Storthing, by 73 votes against 39, has voted a grant of 200,000 kroner for Dr. Nansen's North Pole Expedition.

THE third International Shorthand Congress will be held at Munich from August 7 to 17. The centenary of F. X. Gabelsberger, the originator of modern German shorthand, will be celebrated by those who attend the meetings, and a bronze statue of him will be unveiled.
From *Nature* July 3, 1890.

50 YEARS AGO

GENERAL de Gaulle, leader of the French Volunteer Legion, ha appointed M. André Labarthe director-general of all French se vices connected with armaments and scientific research in Grea Britain. M. Labarthe, who is thirty-eight years of age, has appeale to French engineers, men of science, and industrial workers to joi the already large number of their colleagues who have enlisted a civilian workers in the Legion.

AN instructive address on television given at Buenos Aires last yea by Mr. W. E. Tremain, chairman of the Argentine Centre of th Institution of Electrical Engineers, is published in the May issue o the *Journal of the Institution of Electrical Engineers*. Mr. Tremain begin by saying that the more we study recent developments in television the more we are forced to realize that high-definition television i one of the outstanding scientific and engineering achievements o our time. In August 1936, the Alexandra Palace transmitter i north London was in full operation, giving the first public televisio service in the world. On May 12, 1937, the Coronation processio was televized with much success. It was the first outside televisio exhibition of importance. Mr. Tremain points out that television i destined to play in the future a leading part both as a means o communication and as a means of instruction. When planning fo the future, it is important to bear this in mind. In England, as a result of pressure both from the cinema and radio industries, the experimental work being conducted for the purpose of extending the television service in the provinces was expedited. If a definite decision be taken with this end in view, we can look forward hopefully to it giving much direct employment in happier times.
"Significance of Television".
From *Nature* July 6, 1940.

10 YEARS AGO

THE 1980 general meeting of the International Whaling Commission at Brighton on 21–26 July will have on its agenda a call for a total moratorium on whaling. This is the third time that the issue has been raised; on previous occasions, however (1972 and 1979), the motion was amended to one for selective moratoria. This year, the proposal for a total moratorium is tabled, *inter alia*, by France, the Netherlands and the United States, and is felt to have an even chance of success.

As in previous years, defeat or amendment of the motion would probably result not from a change of heart by the pro-moratorium lobby but from a feeling that adoption of such a motion would push anti-moratorium countries out of the convention and therefore beyond international control.

There has been considerable speculation that, should the pro-moratorium advocates carry the day at Brighton, the Soviet Union and Japan might set up a rival commission of their own. Already the two countries have had some bilateral cooperation over quotas.

When, last year, the Soviet Union accepted the IWC's decision for a partial moratorium, in which fishing from factory ships would be confined to minke whales, it implemented this decision by ceding to Japan its North Pacific quota of 230 bryde whales in return for 300 of the less valuable minke whales from Japan's quota in the Antarctic.
"Whaling quotas: More wrangling".
From *Nature* July 3, 1980

IN NEXT WEEK'S ISSUE

Science in France — a 20-page supplement ■ Spiral galaxies a shade opaque ■ Tracking the Saharan climate ■ Digging deep for seismic reflections ■ Role reversal in insect courtship ■ Fossilized muscles ■ Function of Wilms' tumour gene.

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New PHARMACOLOGY TITLES from Macmillan

Peptide and Amino Acid Transport Mechanisms in the Central Nervous System

Edited by L Rakic, Professor of Neurobiology and Biochemistry, Belgrade Clinical Centre; David J Begley, Lecturer, Department of Physiology, King's College London; Hugh Davson, Professor of Physiology, St Thomas Hospital, London and Berislav, Associate Professor, Belgrade University Institute of Medical Physiology

The way in which the brain regulates its internal environment separately and distinctly from the rest of the body has long generated intense interest and debate. Today, advances are being made at an ever accelerating rate. This volume addresses itself to regulation, within the brain extracelluar fluid, of peptide and amino acid levels, particulary those which are known or putative neurotransmitters and neuromodulators. Particular attention is also devoted to the way in which peptides may penetrate the blood-brain barrier or alter its permeability to other solutes. This volume is of vital relevance to anyone studying central nervous system disease, especially the fundamental problem of designing drugs and agents which will penetrate the blood-brain barrier and excert central nervous actions. The relevance to mental health and disease is also unquestioned.

Contents: Introduction H.Davson Neuropeptide Transport Mechanisms in the Central Nervous System. B V Zlokovic et al Peptides and the Blood-Brain Barrier Penetration and Modulating Influences. W.D Banks et al Structural Aspects of the Blood-Brain and Blood-CFS Barriers with Respect to Peptides and Proteins K Moligard Proteins in CFS of the Developing Brain N R Saunders & K Dziegliewska Cyclic Necleotide Mediated Regulation of Albumin Transport in Brain Microvessels F Joo Peptides and Cerebral Microvessels. M Spatz Transport and Barrier Systems of the Cerebral Microvasculature Enzymatic Aspects B M Duricic & B B Mrsulja Transport of Insulin at the Blood-Brain and Blood-CFS Barriers: L K. Squires & D J Begley Insulin as a Regulatory Peptide in the CNS D.G Baskin et al Experimental Psychosis and Transport of Amino Acids and Peptides Across the Blood-Brain Barrier L.K Rakic et al Transport of Enkephalins from the Cerebrospinal Fluid of the Rabbit D.J Begley Control of Opioid Peptide Levels by Peptidases in the CNS. L B Hersh The Effect of Cyclic AMP and Adenylate Activators on CSF Production S Hyman et al Peptide Receptors of the Cerebral Capillary Endothelium and the Transport of Amino Acids Across the Blood-Brain Barrier A Ermisch et al The Effects of Vasopressin and Related Peptides on Tolerance to Ethanol P Hoffman Sleep Effects of Delta-Sleep Inducing Peptide DSIP: V Susic Techniques for Investigating the Blood-Brain Barrier in Non-Mammalian Species J Abbott & B V Zlokovic Amino Acid and Prolactin Transport into CNS: A Lorenzo Amino Acid Uptake in Synaptosomal Preparations. A Lajtha & E A Debler Exchange Diffusion of Neutral Amino Acids from Blood to Brain. A. Gjedde Kinetic Analysis of Blood-Brain Barrier Transport with References to Amino Acids. J Hargreaves & V Cunningham The Mechamism of Amino Acid Transport Across the Choroid Plexus M B Segal & B V Zlokovic A Comparative View of Amino Acid Transport at the Blood-Brain and Placental Barriers D L Yudilevich & C P.D Wheeler Discussion and Conclusions Index

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Peripheral Actions of Dopamine

Edited By Christopher Bell, Department of Physiology, University of Melbourne, Australia and Barry McGrath, Monash University Medical School, Melbourne, Australia

This book reviews the current state of research in three main areas concerning the actions and significance of dopamine in the periphery: the source and effects of endogenous dopamine; the classification and distribution of, and the effects of activating, various populations of peripheral dopamine; and the therapeutic significance of dopamine receptor activation and renal disease.

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Pharmacology and Functional Regulation of Dopaminergic Neurons

Edited by Philip M Beart, Geoffrey N Woodruff and David Jackson

This is the proceedings of a Satellite Symposium of the 9th IUPHAR Congress in Sydney in 1987 entitled Dopamine 87. It covers all aspects of research into dopamine systems and their regulation.

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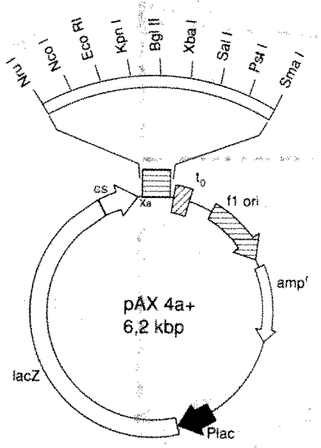

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Nco I	Nco I	Nco I	Nco I
Eco RI	Eco RI	Eco RI	Eco RI
Kpn I	Kpn I	Kpn I	Kpn I
Bgl II	Bgl II	Bgl II	Bgl II
Xba I	Xba I	Xba I	Xba I
		Stop	Stop
Sal I	Sal I	Sal I	Sal I
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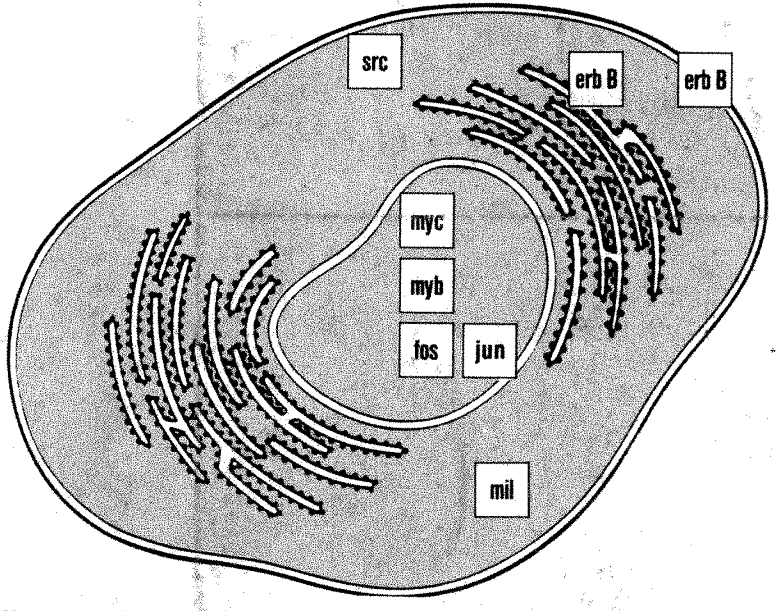

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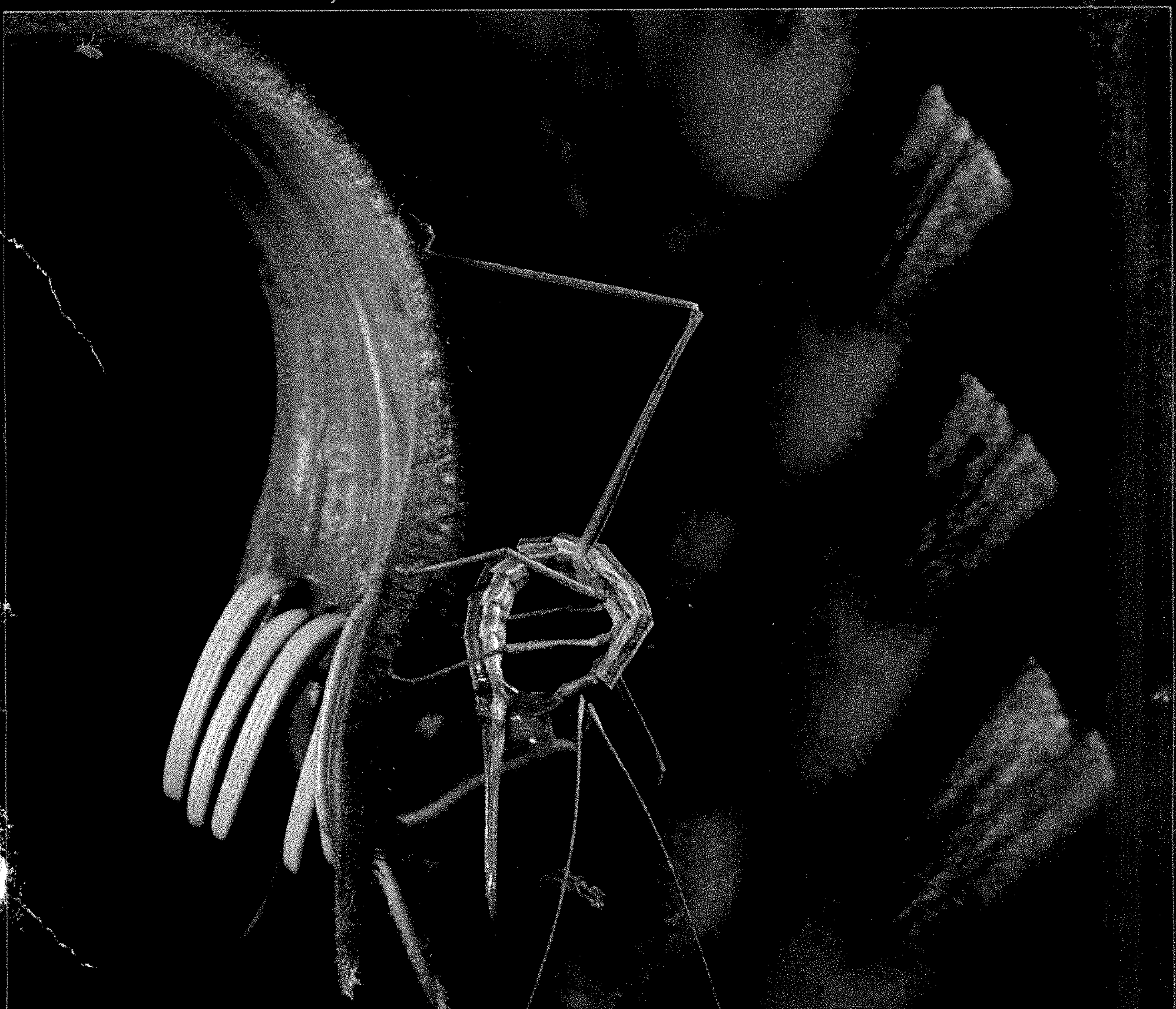

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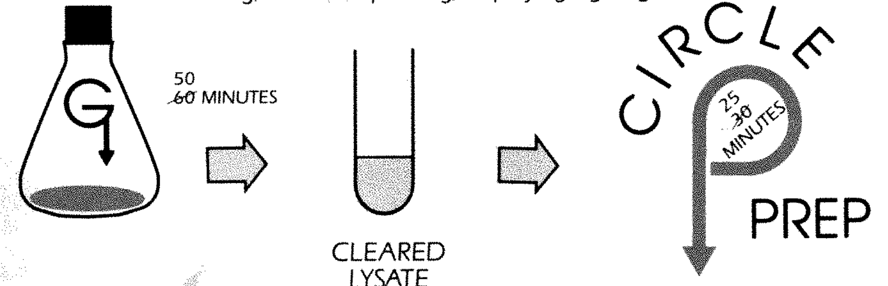

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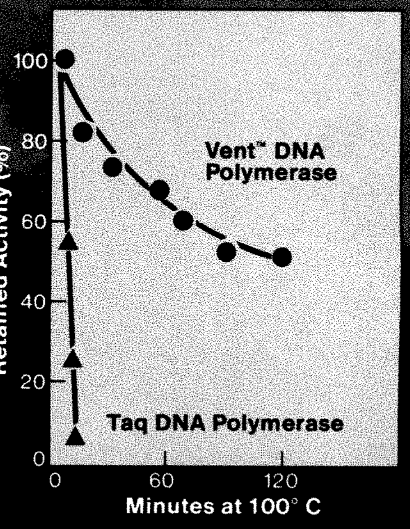

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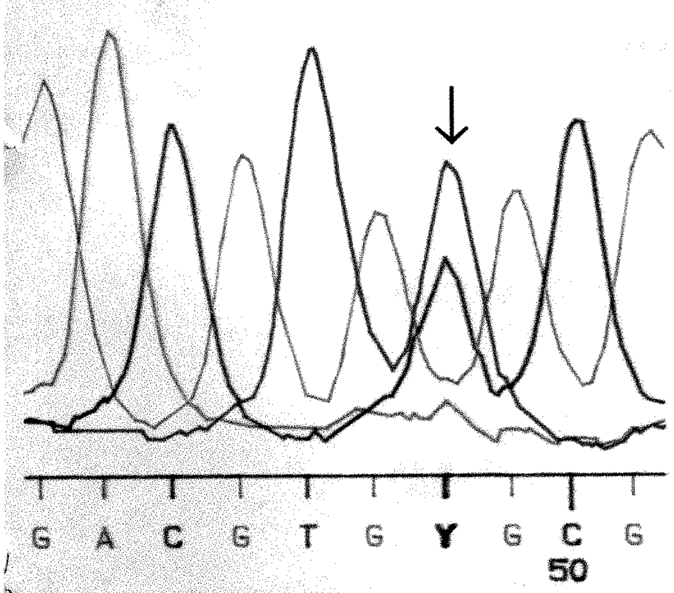

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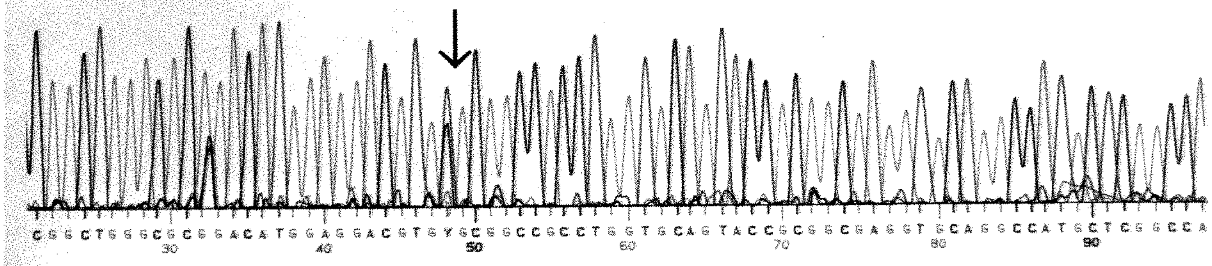

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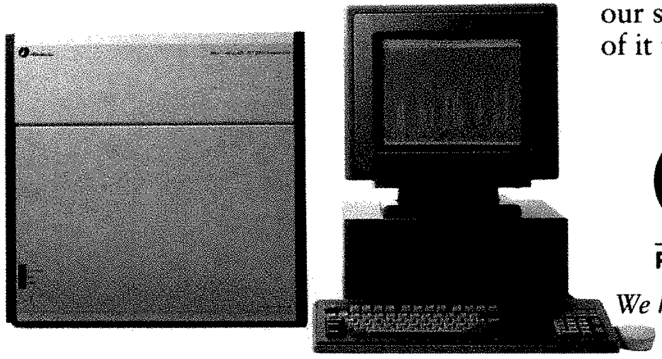

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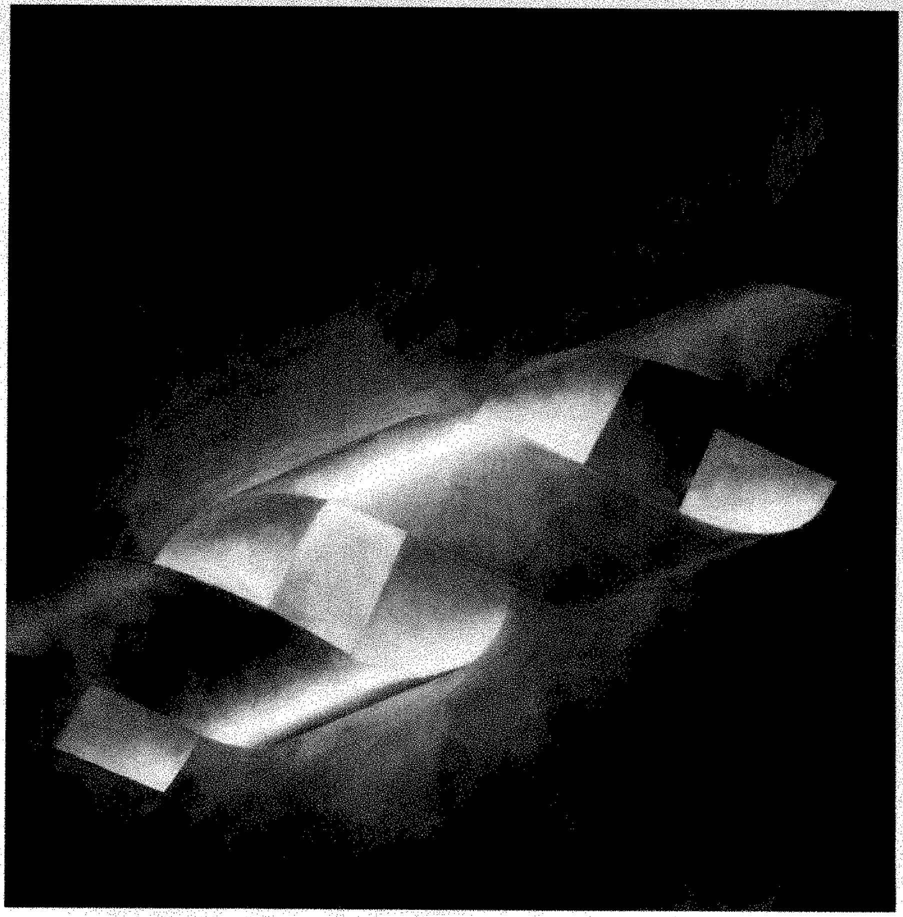

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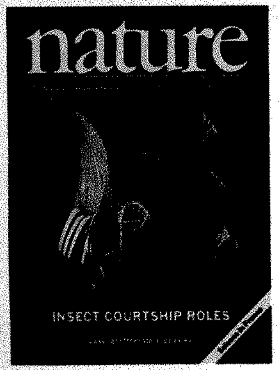

nature

12 July 1990
Vol. 346 Issue no. 6280

◀ In at least one species of katydid, variations in relative male and female parental investment determine the degree of competition for mates, lending support for a central tenet of sexual selection theory. Cover shows a female katydid on a 'kangaroo paw' plant feeding on spermatophore. Page 172. 'Science in France', pages 121–140.

THIS WEEK . . . THIS WEEK . . . THIS WEEK . . .

Dark stars

A survey of surface brightness of 16,000 spiral galaxies as a function of their inclination to the line of sight suggests that they are much more 'optically thick' than was supposed. If this is so, the amount of dark matter in these galaxies may be much less than previously thought. Pages 153 and 105.

Fossils with muscle

Rocks of the Santana Formation in Brazil contain many exceptionally well preserved fossils from the Lower Cretaceous period. In fossilized striatal muscle tissue from the fish *Notelops brama*, electron microscopy (page 171) reveals clear banding in the muscle fibres, and even cell nuclei. Similar details preserved in a pterodactyl wing illustrate the problems of interpreting such fossil evidence, page 116.

Retrovirus infections

Infection of neurons by a murine neurotropic retrovirus which causes spongiform degenerative changes in the brain, and the failure to detect viral envelope protein in infected neurons, raises the possibility that abortive infection of neurons or glia may be a factor in the pathogenesis of human retroviral diseases of unknown aetiology. Page 181.

Wilms' gene expression

The gene whose absence is believed to be responsible for Wilms' tumour is shown by Pritchard-Jones *et al.* on page 194 to be expressed in specific tissues of the developing kidney, which is consistent with the renal tumours and nephritis associated with the mutant gene.

Underground map

The detection of seismic reflections 110 km beneath ground level — the greatest depth so far investigated — paves the way for 'mapping' the base of the Earth's outer shell. Page 165.

Moving pictures

Neurons in the middle temporal area (MT) of the visual cortex show direction-selective responses to stimuli with properties suggesting that they underlie the perception of visual motion. This suggestion is strengthened by the demonstration that microstimulation of MT neurons biases the perceptual judgement of the direction of motion. Pages 174 and 106.

Getting complicated

The change from arid to humid climate in the Sahel and Sahara at the end of the last glacial period may not have been the gradual process assumed by many climate modellers. Pages 141 and 107.

Solid progress

US manufacturers will continue to lag behind Japan in the semiconductor industry — unless they change their ways. Commentary, page 101. On page 155, a step towards polymer-based electronic devices.

Methane controls

Tundra soils contribute 10% of the global atmospheric methane budget, but production of this 'greenhouse' gas would decrease as the soil dried out in the event of global warming. The tundras may thus provide a negative feedback on atmospheric methane. Page 160.

On target

The ability of excess E1a to 'squelch' transcription activated by its own 'activation region', but not that activated by 'acid blob' activation regions, suggests that it interacts with a distinct cellular target factor. Page 147.

Prion knowledge

Can the agents responsible for scrapie and bovine spongiform encephalopathy be transmitted to humans via sheep and beef products? See page 113.

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TB1	20-25
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TABLE #1: Percent rearrangement calculated from the number of cells maintaining a tester plasmid carrying an unstable inverted repeat in its unrewarding configuration (chloramphenicol resistant) divided by the total number of plamid-containing cells.

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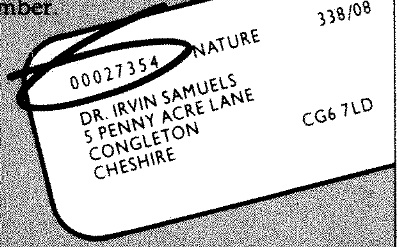

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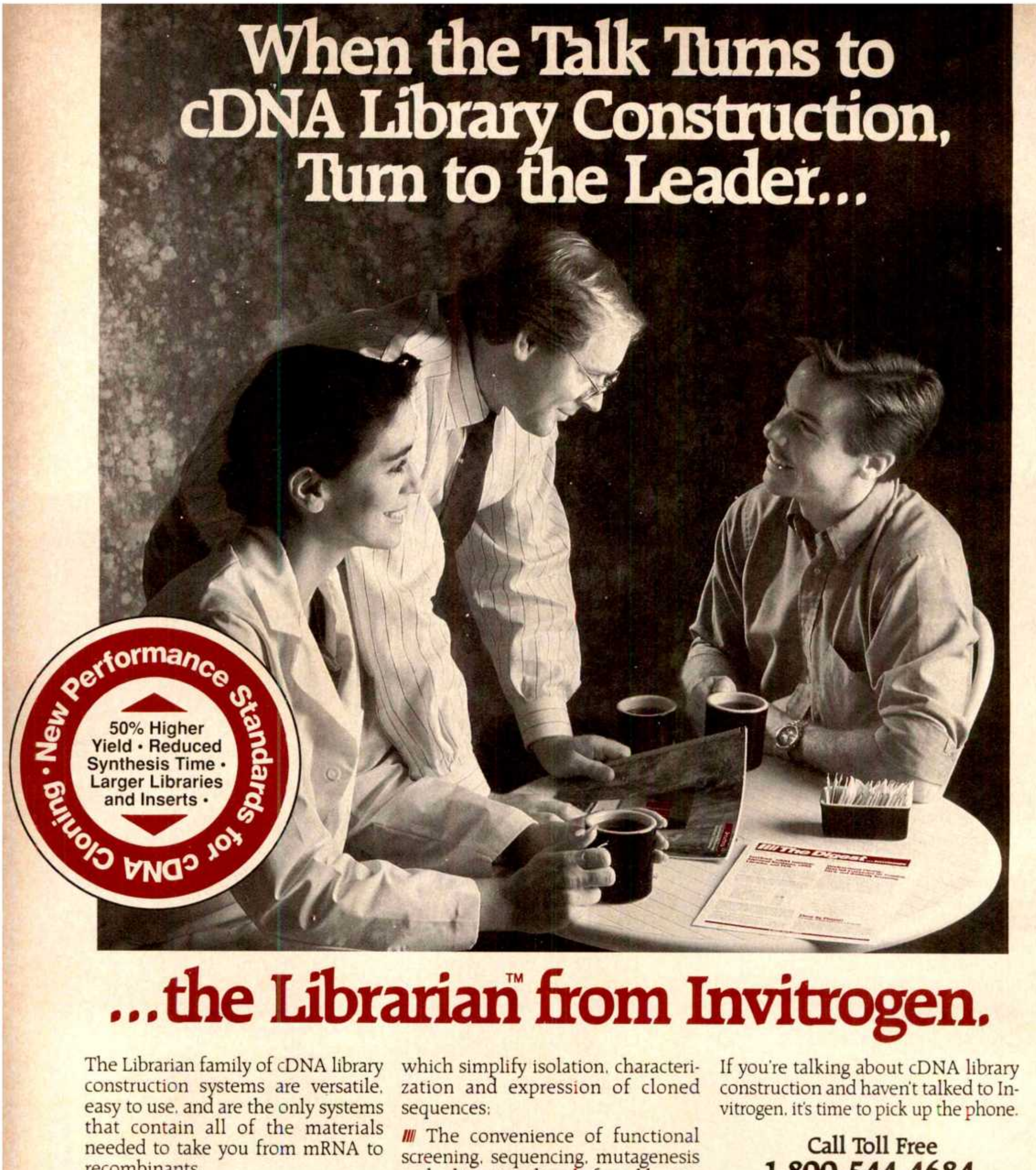
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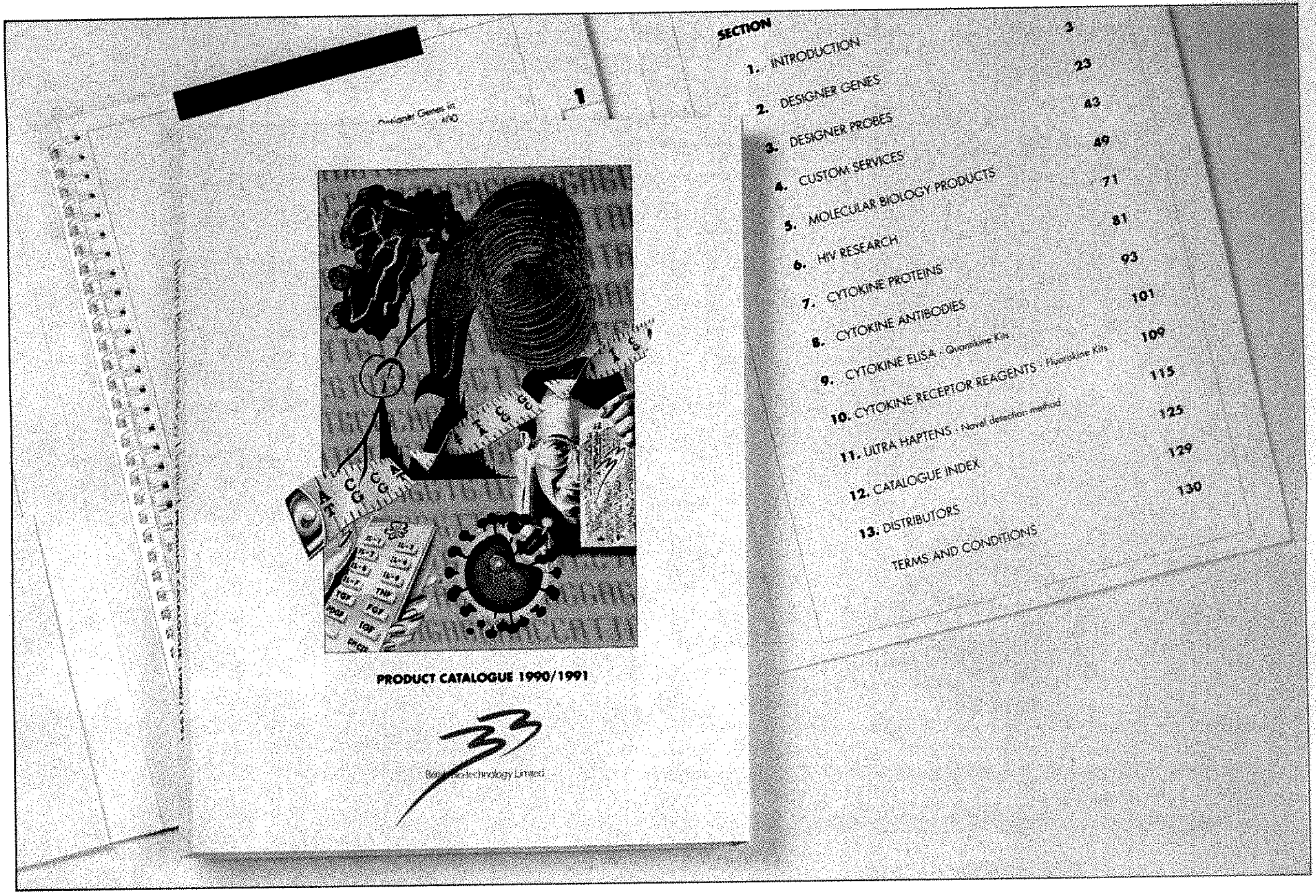

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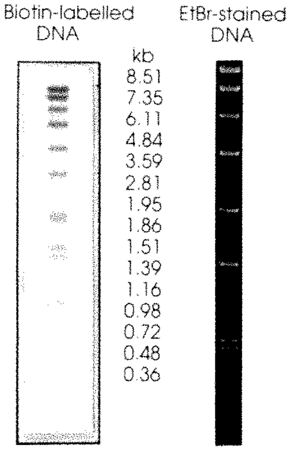

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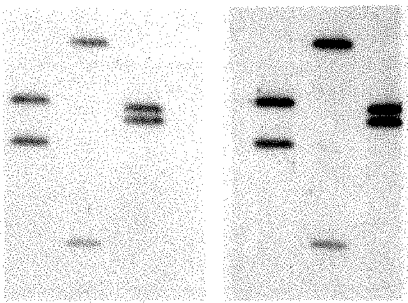

A. Autoradiograph B. Lumigraph^{SM}

DNA fingerprint of 3 genomic samples hybridised with:

A. Minisatellite probe labelled with ^{32}P: Film exposed for 6 hours
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More rows about Europe's money

The British government is making a last-minute attempt to persuade the rest of Europe that it has a better way of creating a common currency. It has a good case, but one likely to be overtaken by events — and by the Deutschmark.

IF people contemplating marriage prudently anticipate as many of the consequences as they can, why should countries planning union be more casual? Mr John Major, the British Chancellor of the Exchequer, could usefully have used that argument when, last week, he launched his last-ditch campaign against the European Communities' plan to legislate for a single European currency. Major says that the plan now proposed in Brussels is a recipe for enormous intra-European resentment. There is no doubt that he is correct; it is obtuse of national treasurers elsewhere in Europe not to recognize as much. This unsolicited expression of support for Major's isolated position is not biased by the circumstance that his own proposals for the future — the coexistence of national currencies with the European Currency Unit (ECU) — coincides with the interim solution advocated by this journal a year ago (*Nature* **341**, 89; 1989).

The correctness of Major's position is well illustrated by the monetary union of the two German states from the beginning of this month. People in the East will now spend a substantial part of their incomes on the products of factories in the West, with the result that many — estimates range from 10 per cent to 25 per cent, but reality could be worse — will be thrown out of work. That prospect is palatable only because the special circumstances promise that West German investment in the East will be so substantial and so quick that the economic upheaval will be short-lived. Let us hope so, for the sake of the civility of the united Germany.

The Brussels plan for European monetary union being pushed by the Commission president, M. Jacques Delors, would effect for the whole of the European Commmunities the kind of monetary union East and West Germany agreed last week, but without comparable compensatory arrangements. It is astonishing that the weaker economies in the European Communities, Greece and Ireland, for example, so casually applaud the Delors plan. Major is rightly worrying about Britain's future.

There may yet be a solution. The meeting of the heads of the European Communities' governments last week in Dublin convened not just one, but two, constitutional conferences in December on monetary and political issues respectively. They should be rolled together, for the second could allow the decisions of the first to be made acceptable. The model here is that of the United States, which enjoys the benefits of monetary and political union within which 50 states retain a substantial degree of autonomy mistaken, in many a state capital, for sovereignty. In practice, the states raise funds from property and sales taxes and take responsibility for education, regional infrastructure and some aspects of welfare, while the federal government uses a nationwide income tax to pay for national defence and supplementary welfare costs. (The costs of federal research agencies such as the National Science Foundation and the National Institutes of Health are peanuts by comparison.)

In Europe, that arrangement is turned upside down. Defence and social security remain national responsibilities, financed by national income taxes, while the nearest thing to a central government (the European Commission) is financed by a sophisticated version of a sales tax, most of which is spent on keeping Europeans working as farmers when they should be doing something else. There is no mechanism by which economic imbalances will be offset by the flow of funds towards the poorer regions. (The European Communities have what is called a social fund, but it is too small to matter and is deliberately marginalized politically.)

Major's proper worry is that monetary union on the basis of fixed exchange rates (the ultimate Delors plan) will rob him and his successors of monetary freedom: interest rates will be determined by the market (and will be lowest in the most productive countries) while the device for repudiating debt called devaluation would be both illegal and impractical. (If West Virginia now sought to solve its economic problems by offering 90 cents for each federal dollar, it would quickly find itself in queer street.)

In the short run, Major is right; let national currencies and the ECU coexist while people get used to the idea, and so that the commercial banks that convert one European currency into another no longer enjoy a right to collect an arbitrary tax on every currency transaction. Further ahead, there can be no monetary union without preceding political union of some kind — at least an understanding of who pays for defence (and thus a common understanding of what defence is for) and a commitment by the rich towards the welfare of the poor. The sad truth about the argument about European monetary union is that there is as yet no serious constituency for either of these causes, and that December is not very far away. □

How did AIDS begin?

What is to be made of a death from pneumonia in 1959, after an apparent immunological collapse?

A THOROUGHLY compelling tale came to light in last week's issue of *The Lancet* — that of how it has been possible, by making use of the technique of the polymerase chain reaction (PCR) for amplifying pieces of DNA, to prove that a seaman who died at a Manchester (northwest England) hospital in 1959 most probably died of AIDS (G. Corbitt, A. S. Bailey & G. Williams, *Lancet* **336**, 51; 1990).

There are two things to say, of which the most obvious is that the report is another vivid attestation of the power of PCR to identify particular nucleotide sequences in tissues long since dead in all clinical senses. On this occasion, by good fortune, some samples of tissue from the dead man had been preserved in blocks of paraffin wax. What now unambiguously emerges is that the kidney, bone marrow, spleen and pharyngeal mucosa (but not samples of liver and brain) carry nucleotide sequences characteristic of the virus believed to be responsible for AIDS, HIV (human immunodeficiency virus). Like Lazarus, it seems, the dead (and their secrets) have been brought to life.

The interest of this development is that it will throw further confusion on questions of the origin of AIDS, of its apparently rapid spread in the past decade and of its long-term potential. The year 1959 is a significant one because it is that from which dates the earliest sample of blood which is known to contain antibodies against HIV, collected in Zaire. So at least two adults died of what would now be called AIDS in 1959. It is unlikely that they were the only victims in that year. And if there were at least two victims in 1959, how many were there in 1958, and 1957 . . . ?

Nobody, of course, can know these things for certain. But the infection appears to have been much the same in 1959 as it is now. The dead Manchester seaman appears to have infected his wife and she the youngest of their three daughters, both of whom afterwards died of a similar syndrome. For what it is worth, the fifties was also the decade during which immunodeficiency diseases came to the fore under the general heading of 'leukaemia'. There was a ready explanation at the time: that previously, without the benefit of antibiotics, people with immune deficiency such as that associated with leukaemia had died of chance infection. It would be surprising if those deaths did not include a sprinkling of AIDS patients. That would agree with estimates of the time since the divergence of HIV from the simian immunodeficiency viruses from which they appear to be derived (see, for example, T. F. Smith *et al. Nature* **333**, 573; 1988).

If there were sporadic AIDS cases during these years, why did the present spread of AIDS in developed countries not begin until the end of the 1970s? HIV is not especially infectious, has a long incubation time, but eventually kills those infected. The sad case of the Manchester seaman shows how an isolated case of infection may run into the sand, with personal tragedy but no spread of infection beyond the immediate family. Given HIV, the rate at which the infection spreads is determined by the rate at which infected people acquire and infect new sexual partners in the interval between infection and death, with the transfusion of infected blood, the use of infected hypodermic needles and maternal transmission amplifying the process. All that is now reasonably well understood, as is the truth that the spread can be controlled only by individual precautions to ensure that sexual intercourse does not lead to infection by HIV and that other high-risk behaviour is avoided. The high incidence of HIV infection reported from many African countries is a sign of the end-point which, it is to be hoped, will elsewhere be avoided. □

Moving the goalposts

Disappointment with the World Cup could be simply prevented in future competitions.

NOT for the first time, the competition called the World Cup has ended with players and spectators alike complaining of their disappointment. One BBC commentator was heard to declare that "This hasn't been the best World Cup in the world". But the faults of these competitions, and of the game of football which is their substrate, are well recognized.

This time, the complaint is that too many matches ended with neither side having acquired a score, so that a "result" had to be manufactured, for the convenience of the organizers, by means of a humiliating game of chance in which people kick a football just beyond the reach of a person called a goalkeeper. But that is a superficial issue. It is much more important that the game of football, as at present played, invariably yields such small scores that they cannot possibly be an objective measure of the relative quality of two teams.

Plainly, the game needs radical redesign so as to increase the likelihood that scores different from zero will result. As things are, it is probably fair to represent teams' scoring potential by a Poisson distribution of the form $P(N) = \lambda^N e^{-\lambda}/N!$, where $P(N)$ is the probability of scoring N in a particular match, when λ will be the mean score against comparable opposition. A team's opponents will presumably have a similar distribution, but with a different parameter, say μ. A football game is or should be an experimental test of whether the parameters λ and μ are significantly different. Karl Pearson's F statistic is the appropriate test, but whatever is done, it is inconceivable that there could be a valid test of quality in less than, say, 12 hours play, which would clog all the television channels. It would be simpler to set the goalposts further apart. □

Commission to keep whaling moratorium

■ Debate to be refought next year
■ Value of scientific whaling disputed

London

THE International Whaling Commission (IWC) decided to keep the moratorium on commercial whaling in place for at least another year at its annual meeting in Nordwijk, the Netherlands, last week. Iceland and Japan failed to win approval from the commission for commercial quotas for minke whales but catches as part of 'scientific research' programmes will continue, despite the opposition of most IWC members.

Iceland had asked for a commercial quota of 200 minke whales in the central north Atlantic Ocean and Japan for 50 in the Okhotsk Sea. The minke is the smallest of the great whales and was the last to be hunted.

The Icelandic and Japanese requests had been expected as the moratorium, which came into effect in 1986, was set for revision this year. But the timetable for revision incorrectly assumed that the procedure by which catch quotas were set would be overhauled successfully by 1990.

All agree that a new system is necessary. Quotas depend on accurate population estimates, and information on whales' rate of reproduction. But these estimates are notoriously inaccurate, so that even catches within an agreed quota might drive a managed stock to extinction.

The IWC's scientific committee has produced a shortlist of procedures that may produce quotas that will protect stocks from overexploitation. But repeated computer simulations of their effects on whale populations are still needed.

The three main whaling nations — Japan, Iceland and Norway — accept that a comprehensive review of the commercial moratorium will have to wait until the 1991 IWC meeting in Rekjavik, Iceland. But Lars Walløe, scientific adviser to the Norwegian delegation, believes the anti-whaling nations will delay the adoption of a revised quota system there.

Irrespective of any political pressure, Phil Hammond, a UK delegate to the scientific committee, doubts if a new quota system will be in place in time for Rekjavik. Scientists are uncertain of the geographical boundaries between genetically distinct stocks of minke whales, and have to develop a quota system that will not threaten individual stocks, irrespective of where the boundaries lie.

Even if the new quota system is ready by next year, the anti-whaling majority of IWC members is likely to vote to maintain the moratorium. The whaling nations may then carry out their long-standing threat to leave the IWC.

The controversial issue of whaling carried out in the name of scientific research is a more immediate concern. The scientific committee, meeting before the full IWC meeting, as usual disagreed over the merit of this year's research proposals. Like the full IWC, the committee is influenced by political considerations, with scientists representing the interests of whaling nations clashing with a committed core of anti-whaling biologists.

Debate over a Norwegian proposal to take five minke whales in the Barents Sea (to add to the 46 taken in the last two years) centred around the 'multi-species model' that Norway says will help the sustainable management of Barents Sea whaling and fisheries. Norwegian biologists plan to combine data on minke whale digestion and energetics with similar data for other species, to model feeding interactions.

But some members of the scientific committee doubt if the whale data, with such small sample sizes, will be useful. Sidney Holt, scientific adviser to the Seychelles delegation, argued that the Norwegians should first develop their model, and examine how the whale data will affect it, before taking more whales.

The much larger and ongoing Japanese programme on Antarctic minkes (see *Nature* **344**, 189; 15 March 1990) was the source of more disagreement. As in the past two years, Japan plans to take 300 minke whales, to estimate the age structure of the population.

Doug Butterworth, from the University of Cape Town, a scientific committee member not attached to any national delegation, presented analyses indicating that the Japanese data may be useful, allowing future quotas to be set with greater accuracy.But again, some biologists argued that the sample sizes are too small to estimate age structure. The committee needs a convincing analysis of the margin of error surrounding the age structure estimates, but is not satisfied that the Japanese have provided this. Although a catch of 300 minkes will not seriously deplete the overall Antarctic population (estimated at about 750,000), some biologists believe that the analysis should precede further catches.

Fukuzo Nagasaki, scientific adviser to the Japanese delegation, agrees that a sample of 300 minkes is "rather small", but says accurate age structure data will be collected in five or six years.

The full IWC meeting asked both Norwegian and Japanese governments to "reconsider" their permits for scientific whaling. Nagasaki says that some minor amendments to the research are possible, but the catch will go ahead. The IWC's constitution allows members to take scientific catches, irrespective of IWC resolutions.

The anti-scientific whaling lobby did gain a small victory, however, when a Soviet proposal to take up to 70 minkes and 30 fin whales in the Okhotsk Sea was withdrawn. The Soviet plan was received poorly by the scientific committee, as the aims and methods of the research were unclear, and no Soviet scientists attended to explain the research. **Peter Aldhous**

Reduction request

London

THE IWC, concerned about the huge catch of dolphins and porpoises in Japanese waters, has asked Japan to reduce its catch of Dall's porpoise to below 10,000 animals a year — the number taken in 1986. A report from the British pressure group, the Environmental Investigation Agency, published last month, estimated that at least 50,000 dolphins and propoises are now killed each year off the Japanese coast.

Since the moratorium on commercial great whale catches came into effect in 1986, the shortage of whalemeat for the Japanese market has made porpoise meat a precious commodity, increasing the Dall's porpoise catch to about 40,000 per year in 1988. Although the 1989 catch was somewhat reduced, at around 30,000 animals, this figure still "must be unsustainable", according to one participant in last week's meeting. The entire population of Dall's porpoise in Japanese waters was estimated at only about 100,000 individuals in 1986.

But the resolution, proposed by a number of anti-whaling countries including the United Kingdom, can at most be described only as a forceful request. Fukuzo Nagasaki, scientific adviser to the Japanese delegation, acknowledges that the Dall's porpoise is a genuine concern. But he offers little hope that the new resolution will be obeyed. Although the Japanese government is trying to reduce catches, Nagasaki says, the fishery operates through a system of licences for boats, rather than fixed quotas.

A quota system would take "a long time" to implement, he says.

The Nordwijk meeting also passed a resolution asking IWC's scientific committee to gather information on the size of small cetacean stocks around the world, with the aim of presenting a report to the UN conference of environment and development in 1992. Peter Aldhous

SILVER SPRING MONKEYS

Then there were four

Washington

THE long war over the fate of the 'Silver Spring' monkeys entered a new phase last week when three of the remaining seven monkeys kept at the Delta Regional Primate Research Center in Louisiana were killed after a series of experiments were carried out on their brain activity. As in the past, neither side in the fight over the fate of the monkeys was willing to soften their rhetoric. Both the animal rights groups and the congressmen who regard the monkeys as symbols of laboratory mistreatment of animals, and their opponents in the research community and the National Institutes of Health (NIH) continued to denounce each other's motives.

The office of Congressman Robert Roe, which tried unsuccesfully to have experiments delayed until this week in order for Roe to discuss the monkey's fate with William Raub, the acting head of NIH, was scathing in its criticisms. Gregory Simon, staff director in Roe's office, described NIH's decision to kill the monkeys as "a vulgar display of muscle", which appeared to be designed "merely to make the point that the scientific community is totally in control of animal research and will not let anybody else influence the way it is done". Roe's office threatened public hearings on NIH's conduct and renewed efforts to pass a bill that would force NIH to send the remaining monkeys to an animal sanctuary.

People for the Ethical Treatment of Animals (PETA), the animal rights group which has made the monkeys into a public issue, erected a protest sculpture, depicting the 'torture' of a monkey on a laboratory apparatus, outside the Department of Health and Human Services. PETA reminded the press that the monkeys are still the biggest cause of public appeals to the White House, claiming that it had received 45,000 letters last month asking Barbara Bush, the wife of the president, to free them.

But NIH continues to point out that it has acted correctly and according to the demands of the law. Raub said that it had sought permission to kill the three animals after their health deteriorated and veterinary experts had recommended euthanasia. "The department has made its intentions known for a long period," says Raub, "once the court matters were cleared up it was our obligation to proceed. A delay for an arbitrary period of time would be totally contrary to the representation we made to the court, Congress and everyone else."

Animal rights groups and NIH have agreed on virtually nothing from the start fo the Silver Spring saga. According to PETA, when police recovered 17 of the crab-eating macaques from the Silver Spring laboratory of Edward Taub in 1981, they found evidence that the animals had been mistreated. Taub, they claim, was later convicted of cruelty to animals in a Maryland court. Supporters of Taub disagree, pointing out that Taub was cleared of all charges on appeal and further exonerated in independent investigations.

The latest twist in the saga came after the Physicians Committee for Responsible Medicine, another animal welfare group, failed after a long period of court action to obtain an order to block the euthanasia of three of the monkeys.

Alex Pacheco, spokesman for PETA, says that for "NIH to paint this as a mercy killing is just another lie". He believes that the monkeys were used in experiments and then killed so that NIH could justify having refused for years to release the animals to a sanctuary. PETA claims that the experiments are bogus and points out that in 1985, Raub, who was then Deputy Director of Extramural Research at NIH, wrote that "we have no research protocols, ongoing or planned, for which these animals are appropriate".

Raub says that "it has been explained many times over that the letter was a response to a very narrow proposal". Since then times have changed and the monkeys have emerged as an extraordinarily valuable resource, not for the experiments on rehabilitation from injury which were originally planned, but to test new hypotheses concerning the degree to which the brain is able to reorganize itself after inputs to specific regions are lost. The Silver Spring monkeys had had nerves cut in their forearms.

Experiments conducted on the three monkeys before they were killed revealed "massive reorganization" in the cortex, according to Timothy Pons of the National Institute of Mental Health who lead the team. The data seem to confirm those gained from experiments carried out on one of the monkeys which was killed earlier this year (see *Nature* **343**, 581; 15 February 1990). With data from four monkeys in hand, Pons says he will prepare a scientific paper for publication.

The significance of the science is not really the problem bothering Roe, who is chairman of the House of Representatives Committee on Science, Space and Technology, so much as the way in which the sensitive issue of the monkeys has been handled. Roe's staff members have spent the last six months, according to Simon, "working with PETA and with NIH to come up with the middle ground — an independent panel that would review the disposition of the monkeys". When agreement had apparently been reached, Simon claims that NIH changed its mind. "Once they won in court everything changed and we're talking strictly 'might is right'", says Simon.

But Raub says that it was incorrect to say that NIH backed out of the negotiation. He said he had found it very "disheartening how little progress we made and how far apart the views of the animal rights groups and the NIH were. As we were making little progress there and the court restraints were being resolved a hundred per cent in our favour, and these animals had been judged by experts as candidates for euthanasia as far back as 1988, our obligation under the Animal Welfare Act was that we had to proceed with [the euthanasia] of those three animals".

Alun Anderson

AUSTRALIAN SPACEPORT

Soviet rockets gain US go ahead

Washington

PROSPECTS for the Australian Space Base at Cape York in northern Queensland are once more looking bright after US President George Bush indicated last week that he would not prevent US commercial satellites from being launched on Soviet-built rockets.

A major problem for the base has been that its likely customers were US companies wanting to launch US-made satellites while its cheapest source of launch vehicles lay in the Soviet Union. Until last week, the United States had objected to Soviet launches for US satellites, partly because they feared 'unfair' competition for domestic launches from state-subsidized Soviet organizations and partly because they feared that sensitive satellite technology might get into the hands of Soviet technicians.

The Cape York base, which will lie conveniently close to the equator, will cost around A$350 million to build and could be ready for its first launches in 1995. The project is entirely commercial and is backed by a large Australian property developer, but the contract for running the base seems likely to go to a US company or to British Aerospace. It is not yet certain that the spaceport will be built, as environmental impact studies have yet to be completed and objections from aboriginal groups considered (see *Nature* 343, 400; 1 February 1990).

Tougher competition for satellite launches is bound to follow the entry of Soviet vehicles into the field, alongside Arianespace which is already well established, and the several US companies that are just beginning to provide commercial launches. A controversial US decision made last year also gave limited rights to the Chinese to launch US satellites.

Alun Anderson

AIDS POLICY

Slanging match with Sullivan

Washington

RELATIONS between the US government and AIDS activists took a turn for the worse last week when Health and Human Services Secretary Louis Sullivan released a statement calling on health officials to avoid all but "necessary and productive" dealings with the radical protest group ACT-UP (AIDS Coalition to Unleash Power).

The statement is Sullivan's response to a noisy demonstration, led by ACT-UP, which drowned out his closing speech at last month's International Conference on AIDS in San Francisco. Upset by the attack, during which he was pelted with wads of paper and other makeshift missiles, Sullivan now says that he will not "let [his] department be used as a punching bag" or "have dealings with people who behave as ACT-UP did".

Peter Staley, a spokesman for ACT-UP, says that initially his organization took Sullivan's statement "very seriously". If the threats implied by this "politically naive" statement were carried out, says Staley, "it would be tantamount to a decaration of war".

But the Secretary's bark may well prove bigger than his bite.

When pressed for details, James Brown, a spokesman for the Public Health Service, was unable to give specific examples of the kinds of dealings with ACT-UP that will be curbed, although he said that the positions currently held by members of the group on research advisory panels are not in jeopardy. The Secretary will not stop "certain programmes" from drawing on ACT-UP's knowledge and expertise, Brown said.

ACT-UP says that so far there has been no evidence of any pressure on their many contacts in the Food and Drug Administration and National Institutes of Health (NIH) to stop communicating with its members.

Sullivan's words come at a time when activists and public health officials, particularly at the Food and Drug Administration and NIH, are nurturing a growing relationship. Members of ACT-UP currently sit on two of NIH's research advisory panels and expect to be awarded more committee places when the AIDS Clinical Trial Group at the National Institute of Allergy and Infectious Disease, which oversees the bulk of US AIDS research, is opened up to community representatives later in the year. "We certainly hope those won't be jeopardized", says Staley.

David Concar

AIDS EPIDEMIOLOGY

Another early case identified

Washington

THE date of the first known death from AIDS has been pushed back to the 1950s. Researchers at the medical school of Manchester University, England, have found evidence of HIV (human immunodeficiency virus) genetic material in tissue specimens from a 25-year-old caucasian seaman who died of severe pneumonia in Manchester in 1959.

Previously, the earliest recorded cases of HIV infection, identified retrospectively, were in three members of a Norwegian family who contracted AIDS in the 1960s and died in 1976. HIV antibodies have also been found in a blood sample collected from a patient in Zaire in 1959 — but in that case there is no evidence that the patient died from AIDS.

The new finding will not initiate a rethink in the epidemiology of HIV infection. According to Roy Anderson of Imperial College in London, epidemiological analysis of high present-day infection rates in places such as Malawi indicates that HIV must have been present in Africa for at least 30 to 40 years. More reports of early sporadic cases of AIDS will emerge, says Anderson, as researchers look back over the records. He also emphasizes that the Manchester seaman represents an unlikely point of origin for the current AIDS epidemic in Britain.

To obtain evidence for HIV infection, the Manchester researchers used the polymerase chain reaction (PCR), a technique that allows trace amounts of genetic material — in this case proviral DNA — to be amplified so that its identity can be determined using a DNA probe. One drawback with PCR is its sensitivity to contaminants, a problem which in the past has led to false results. Gerald Corbitt, one of the three researchers who reported the case in the British medical journal *The Lancet*, says that every precaution was taken to prevent contamination of tissue samples. They performed the analysis in a "remote" laboratory and their PCR assay gave the same result when repeated on different tissue samples.

The researchers do not yet know whether the patient was infected with HIV-1 or HIV-2, but are planning to sequence the proviral DNA to find out. Their main limitation is a dwindling supply of genetic material.

Sadly, there is little information on the seaman himself — his earlier medical history and the countries he visited before 1959 are apparently unknown.

David Concar

BIOLOGICAL WEAPONS

Lawyer talks tough

Boston

IN the coming months, many biomedical researchers are likely to receive a disturbing memorandum in their mail, warning them that their research could be breaking the law and they they could be punished by imprisonment for life. The memorandum will come from the office of Francis A. Boyle, a professor of international law at the University of Illinois at Urbana-Champaign who was instrumental in drafting recently passed legislation which incorporated provisions of the international Biological Weapons Convention into US domestic law (see *Nature* **345**, 192; 17 May 1990).

Boyle's memorandum, addressed to "all life science researchers" and circulated first to several scientific publications including *Nature*, alerts researchers to the provisions of the Biological Weapons Anti-Terrorism Act of 1989, Public Law 101-298. In effect, the law makes it a federal crime for a US citizen "knowingly" to develop, produce, stockpile, transfer, acquire, retain or possess "any biological agent, toxin, or delivery system for use as a weapon", or to aid a foreign state or any organization to do so.

Chuck Dasey, spokesman for the Army's Medical Research and Development Command, says that the Army has abided by the Biological Weapons Convention since its inception and thus by definition abides by the new legislation as well.

In a telephone interview last week, however, Boyle stated that the Army's contention is precisely what may now be challenged through either civil or criminal lawsuits involving specific research projects. In his memorandum to researchers, Boyle states that in his "professional opinion" many research projects that have already been funded by the US Department of Defense's Biological Defense Research Program (BDRP) "raise serious compliance problems" with the new law. Consequently, Boyle urges researchers who are receiving or applying for funding from the BDRP to "obtain legal advice and counsel from a competent attorney" about their research or grant application.

Boyle is not shy about his opposition to the BDRP programme. He stresses that in his view, "the first step is to warn researchers about the law" in the hope that they will look carefully at whether their work is fully in compliance. For researchers who continue to receive military funding, however, Boyle acknowledges that he may be personally involved in a legal challenge against them in the future if their work appears to him to stand in violation of the law.

Seth Shulman

HIPPARCOS MISSION

And now some good news

Paris

AFTER 11 months of calculation and rethinking, scientists at the European Space Agency (ESA) now believe that the seemingly ill-fated astronometry satellite, Hipparcos, will be able to perform its mission successfully after all. A request for ESA to build a follow-up, Hipparcos 2, has been dropped.

Hipparcos was launched last August by an Ariane rocket with the intention of gathering information on the position, proper motion and distance of 120,000 stars to a new order of precision. The launch itself was perfect — but then the apogee boost-motor failed, leaving the satellite in a highly elliptical orbit, rather than the geostationary orbit that was originally planned (see *Nature* **340**, 581; 1989). The load thus put on the satellite's three solar arrays seemed likely to limit the mission to a duration of six months, rather than the planned four years. Data collection also became a problem as the elliptical orbit put the satellite out of reach of a single tracking station and transmissions were regularly obscured as the satellite passed through the Van Allen belts.

Since then, help to track Hipparcos has been found at ground stations in Perth (Australia), Kourou (French Guiana) and Goldstone (United States), adding to the planned activities at Odenwald in West Germany. That means that the satellite is visible 93 per cent of the time and useful observations can be made 60 to 70 per cent of the time.

The future of the mission depends on the rate at which voltages from the solar panels decline. Mike Perryman, Hipparcos project scientist at ESA, last week said that projections based on rates collected over several months suggest Hipparcos may last two to three years "or four or even longer". That will allow the 120,000 stars of the input catalogue to be positioned with 10–20 milliarc accuracy, still, according to Perryman, 10–20 times better than could be achieved from the ground.

For Perryman, the effect of the failure is now on the quantity of observations, not their quality. "We cannot retrieve quite as much data, but for the 70 per cent of the time when we do get data, the quality is outstanding. We are delighted." Over 5 million observations have already been made of the 120,000 stars.

An 11-man enquiry board set up to investigate the failure of Hipparcos submitted its final report and recommendations last week. French defence department missile expert Daniel Reydellet, who chaired the inquiry board, said in Paris that an 'off-the- shelf' component in the ignition circuit of the apogee booster motor — the through bulkhead initiator (TBI) — apparently did not work. But Reydellet was reluctant to say whether the TBI, made by the California-based company, Hi Shear, was faulty. The TBI depends for correct functioning on pulses from other parts of the ignition circuitry.

The report neverthless suggests that Hi Shear's reputation with the US National Aeronautics and Space Administration may have dazzled ESA engineers into foregoing quality control checks. When, as part of the inquiry, experts visited Hi Shear, Reynellet said they found that TBIs originating from the same batch as the one used in Hipparcos had "disappeared", thereby ruling out fresh tests. Because the components can be used only once, statistics from the testing of a whole batch are needed to assess reliability. Neverthless, analyses being carried out by ESA have suggested that quantities of explosive may have been only just within the required specifications.

Wise after the event, the inquiry board has recommended that, when off-the-shelf components are bought in future, ESA engineers should monitor manufacture more closely. But, given the complexity of modern satellites, this may not be realistic.

Peter Coles

EUROPEAN SPACE PROGRAMME

Columbus shipwrecked?

Munich

A SMOULDERING dispute over the West German space programme flared into life again last week when the 1991 budget of the West German Research Ministry (BMFT) was announced. Space research and technology accounted for half of the 4 per cent increase in the budget, prompting renewed criticism from the opposition parties that BMFT was taking money away from other areas by investing so much in manned space flight.

With the estimated cost of the European space station Columbus, which is supposed to complement the US space station Freedom, having risen by thousands of millions of dollars, West Germany has found its role as main developer of Columbus, bearing 38 per cent of the cost, increasingly difficult to shoulder. When the European Space Agency (ESA) council decided in 1987 to begin three linked space projects — Columbus, the space shuttle Hermes and a heavy payload rocket Ariane-5 — the total cost was estimated at DM 21,000 million ($12,800 million).

Since then, the price has risen to an estimated minimum of DM 30,000 million, according to opposition members of the West German parliament. BMFT refused to confirm any such figure, but admitted that large increases would be necessary in 1992 and beyond to cover the rising cost of the programme. West German Research Minister, Heinz Riesenhuber (Christian Democrat), has promised not to let space research take more than 22 per cent of BMFT's budget. That proportion is now 20 per cent.

Columbus is meant to be both a vehicle for basic research in microgravitation and a platform for industrial research and development, but critics claim that neither goal will be met by the current plan. Opposition Social Democratic parliamentarian Edelgard Bulmahn doubts that the latest US plan for space station Freedom — to use it as a stopping-place for missions to the Moon and Mars — is compatible with using Columbus for microgravity research.

Anticipating a major budget battle, BMFT requested as early as 1987 that ESA cut its budget for the three projects by 15 to 20 per cent. But Buhlman says that cuts of this size would reduce the amount of new technology that European countries could develop, forcing ESA to buy much of the needed technology from the United States.

The members of Helmut Kohl's centre-right coalition gave a vote of confidence to Columbus last month before parliament adjourned for the summer. But the support may be conditional. Christian Democrat parliamentarian Christian Lenzer says he is "totally against building something that industry is not interested in using," sowing the seeds for a conflict with BMFT, which defends the project as an important investment in basic research. And a spokeswoman for the prime contractor for Columbus, MBB-ERNO in Bremen, said that "it will take 15 years" before the space station "becomes a business" the way that satellites or launch vehicles have.

Adding to the confusion, an ESA review board gave the MBB-ERNO proposal a low rating; the proposal was judged to be "of a generally low level of quality", and the "quality and accuracy of the cost data" was declared "totally unacceptable, with an extremely low level of credibility". An ESA spokesman said it was normal for a large proposal to receive a negative evaluation early in the approval process.

Next year, ESA member states will meet to decide how to proceed with the three space projects. If costs rise more than 20 per cent from original estimates, member states have the option to withdraw from the programme.

Steven Dickman

SPACE SHUTTLE

More trouble for high fliers

Washington

THE temporary disablement of two space shuttles by leaks in the hydrogen feeder lines between the external tank and the orbiter is almost certain to bring extra delays to one or more long-planned astronomy missions, adding to the pain caused by the revelations of manufacturing flaws in the Hubble Space Telescope (see *Nature* **346**, 3; 1990).

Astro-1, an ultraviolet and X-ray astronomy package (see *Nature* **345**, 375; 1990), is waiting for space shuttle Columbia to be repaired after a hydrogen leak was detected shortly before launch. The Gamma-Ray Observatory (GRO) is supposed to fly on shuttle Atlantis in early November, but Atlantis is now stranded on the launch pad at the Kennedy Space Center, Florida. In the meantime the joint US-European solar probe Ulysses must be launched by Discovery in October so that Jupiter is in the right place to give it a gravity-assist into a polar orbit around the Sun. Even if Colmubia or Atlantis were fixed in the next few weeks, the chances are high that Astro-1 or GRO would have to be postponed to let Ulysses fly on time.

The leak in Atlantis was discovered after NASA (National Aeronautics and Space Administration) mission controllers decided, in the light of their experience with Columbia, to conduct a pre-launch 'tanking test' in which the external tank was filled with hydrogen as it would normally be just before launch. They detected a small hydrogen leak, and postponed the Atlantis flight while it and Columbia were investigated. By this time, the various 'umbilical' pipes connecting Columbia to its external tank had been taken apart and tested individually, but the leak could not be reproduced.

Last weekend, NASA engineers tested Columbia's reassembled umbilical system at the Rockwell Downey company, near Los Angeles, where they were built.

This week, Atlantis was being prepared for a new launch-pad test, in which various parts of the umbilical will be surrounded by sealed bags in order to determine which part of the assembly is leaking.

How the launch schedule will be put back together depends on the amount of work needed to fix the leaky sections. If repairs can be made on the launch pad, Atlantis and its secret payload would be sent off as soon as possible, which would probably delay the launch of Astro-1 until after Ulysses. If Atlantis has to be rolled back off the launch pad to be repaired, Columbia and Astro-1 could be launched instead, but then GRO could not be launched until Atlantis's Defense mission had been completed.

David Lindley

LAWRENCE LIVERMORE LABORATORY

Bargain hunters' paradise in California

Washington

LAWRENCE Livermore Laboratory may be one of the largest US nuclear weapons research and development laboratories, with a strong reputation for its 'Star Wars' research, but it sounds a lot more like the thieves' bazaar of old Baghdad in the just published report of an investigation by the General Accounting Office (GAO).

The report says that equipment worth more than $45 million has vanished from the laboratory, which is managed by the University of California, including 49 microcomputers, 205 typewriters and word processors, 841 pieces of video equipment, 388 cameras and related equipment and thousands of calculators. Where has everything gone? A statement put out last week by the office of congressional strong man John Dingell, chairman of the House of Representatives Energy and Commerce Committee and its oversight and investigations subcommittee, noted that allegations had been made at a congressional hearing that "laboratory equipment, such as computers, was stolen and traded for drugs". Dingell, who ordered the GAO investigation, claims that at the laboratory "[w]e have seen drug use, cover-ups, retaliation against concerned employees, poor safety and poor security".

The Department of Energy (DoE) disagrees. In a reply, DoE diplomatically thanks Dingell for his interest, saying in a statement from its San Francisco office that it "consider[s] the findings helpful to improve property management at the lab" and that "improvements are under way". But it also says the laboratory has already located 98 per cent of the 'missing' property. According to DoE spokeswoman Caroline Powell, the San Francisco office has checked that the property is really in place. She says of the GAO failure to find many items, "maybe the thing was in a desk draw and they didn't ask the person who was responsible".

GAO has yet to report on a separate investigation into whether any nuclear materials or classified documents are missing from the laboratory. Dingell has promised to hold hearings later this year to examine the report and DoE's claims.

Alun Anderson

EUROPEAN SCIENCE

New Framework row

London

PLANS by European Community (EC) research commissioner, Filippo Pandolfi, to concentrate EC basic science spending almost entirely on postdoctoral fellowships have set him at odds with member states' representatives on CREST, the EC's scientific and technical research advisory committee. The disagreement could delay spending on basic science through the Framework programme, which supports all research and development efforts in the EC.

EC funds for basic science are set to increase under the third Framework programme, which will run from 1990 to 1994. The increase itself has caused apprehension in some members of the UK research councils, who fear that UK spending through the EC will be taken from the domestic British science budget (see *Nature* **345**, 376; 31 May 1990). But Pandolfi's decision to initiate a 'human capital and mobility' programme which would support several thousand postdoctoral positions has attracted wider opposition.

Only Italy, the present incumbent of the EC presidency and anxious to avoid rifts, has so far not come out against Pandolfi's plan.

Pandolfi argues that promoting the exchange of young scientists between EC member states is useful because it is not strongly supported by individual states. But CREST is concerned that there simply will not be enough young scientists to fill the places, and in any case wants a more diverse programme, with support for postgraduate scientists and large research institutes.

Although the proposed 518 million ECU (1 ECU = £0.70) human capital and mobility programme may include some support for applied science, it is envisaged that it will largely take over from Framework's SCIENCE programme, which is now the centrepiece of EC basic research support.

Supporters of the present diversified system point to the continued success of the SCIENCE programme's research projects, particularly the huge collaborative project on NdFeB alloy-based magnets (see *Nature* **338**, 734; 1989), which has given Europe the lead in this area. These large-scale projects do, in any case, support a number of postdoctoral researchers.

The current SCIENCE programme is due to end in 1992, but Pandolfi says he plans to fund a continued programme up to 1994. This will depend on additional money becoming available, however. Sir Peter Swinnerton-Dyer, chairman of the committee that allocates funds under the existing SCIENCE programme, is concerned that there could be a delay in getting the new programme under way, if CREST and Pandolfi cannot quickly reach a compromise.

Peter Aldhous

EUROPEAN NUCLEAR POWER

Not in my back yard

Munich

As the barriers between Eastern and Western Europe come down, some nuclear power plant operators in the West are wondering if they may not now have a golden opportunity to set up some of their unpopular generating facilities next door. The Czechoslovak government has already begun negotiations with French and German companies to build plants at the Temelin site, near the Austrian border. The plants would be financed by selling electric power to Italy, transmitted via high-tension cables running through Austria.

The idea of building new nuclear plants in Czechoslovakia is controversial, especially in neighbouring Austria, which abandoned its own nuclear programme in 1987. Anti-nuclear activists based in Austria have been campaigning for years against existing Czechoslovak reactors.

Nevertheless, there are clear benefits for Czechoslovakia in expanding its nuclear programme in this way. It could gain access to important Western technology and at the same time reduce its dependence on high-sulphur coal, which has devastated the environment (see *Nature* **344**, 91; 1990).

Czechoslovakia currently derives 27 per cent of its electricity from nine Soviet-built nuclear reactors at three sites. Another seven reactors that are under construction will raise dependence on nuclear power to 45 per cent by the year 2000. The design of two reactors, at Bohunice near the Austrian border, has been criticized as fundamentally flawed. They are of the same type (VVER 440-V 230) as those at Greifswald, East Germany, which were last month judged to be hopelessly unsafe by a West German safety commission and ordered to be shut down. There are four reactors of the same type still operating in Bulgaria and four more in the Soviet Union (see *Nature* **344**, 282; 1990). The remaining seven Czechoslovak 440 MW reactors are of a slightly more modern design.

So far, the newly elected democratic government of Czechoslovakia has continued its Communist predecessor's support for nuclear power. As recently as 27 June, an adviser to the now-defunct Ministry for Fuels and Energy, Frantisek Suranski, declared on Austrian television that Czechoslovakia "sees no reason or option to give up nuclear power now". Even the activist Environment Minister for the Czech republic, Bedrich Moldan, has said that he favours the continued operation of existing nuclear plants and the completion of plants under construction.

Prime Minister Marian Calfa announced on 3 July that several coal-burning plants in northern Bohemia would be closed by the end of the year. That decision will put more pressure on nuclear plants to fill the resulting energy gap. A moratorium declared in January by the interim government on construction of the two newest 1,000 MW plants at Temelin does not mean that nuclear power is out of favour — these are the plants that the government may replace with new Western designs.

Austrian objectors to nuclear power have made several arguments against Czechoslovakia's plans to expand its nuclear capacity. They point out that no one has studied the real cost of Czechoslovak energy or how much Czechoslovak industry could improve its energy-efficiency. According to Michael Undorf of the Austrian Greenpeace organization, the Austrian government has offered to form a joint commission to study this problem, and Moldan has tried to get the Czech government to accept, but without result.

Once Czechoslovakia carries out such studies, said Vienna University ecology professor Bernd Lötsch on Austrian television on 27 June, the Czechoslovak government will realize how much more effective it is to improve the efficiency and environmental standards of industry and existing power plants than to build new nuclear plants. Undorf agrees, saying "We hope that [the prohibitive cost of new plants] is what ultimately defeats the idea."

But conservation alone will not solve the Czechoslovak energy problem, according to Thomas Horacek, a researcher in an institute attached to the former Czechoslovak Energy Ministry. Czechoslovakia is a net importer of electricity from other Eastern European states, and more electricity will be needed relative to other forms of energy as the country modernizes its economy, he says.

Michael Schneider of the Paris-based World Information Service on Energy points out that schemes to finance new nuclear plants by exporting electricity have never worked in the past. Western nuclear power plant producers came close to realizing a deal with Turkey between 1983 and 1987. In exchange for a complete plant built by a Western company, Turkey was to have exported power for 15 years. But by 1987, both West German and Canadian companies had pulled out of the scheme.

If Italy does not in the end prove a willing customer, it is difficult to imagine there being any other market for electricity in another nearby country. Before German unification began, both West Germany and France were producing electricity surpluses, and by the time Czechoslovakia is in a position to build new plants, West German utilities are likely to have met the entire demand in what is now East Germany. Environmentalists have already vowed to fight if Austria moves towards approving the construction of power lines to Italy from Eastern Europe.

Steven Dickman

NUCLEAR CLEAN-UP

Runaway costs cause sharp reduction

Boston

Estimates of the cost of the first phase of the immense project to redress environmental damage occurring at US government facilities used to manufacture nuclear weapons have doubled in less than a year, according to projections released last week by the Department of Energy (DoE). The first five years of the 30-year clean-up are now calculated to require more than $28,000 million. The entire clean-up operation is expected to cost in the vicinity of $100,000–$200,000 million dollars over the next few decades.

DoE says that the projected costs are rising because of a better assessment of the job's technical requirements, and because of the increases in charges that are demanded by contractors to protect themselves against the lawsuits that may arise during the clean-up procedures. Provisions in current hazardous waste laws, as well as increases in environmental litigation by the states and the Environmental Protection Agency, conspire to make contractors increasingly wary, according to Leo Duffy, director of DoE's environmental management office.

Responses to the new cost estimates were mixed. While the Energy Department continues to win high marks for the *glasnost* fostered under Energy Secretary James Watkins' watch, critics complain that the department is absorbing the costs of cleaning-up into operating budget as a way to maintain personnel numbers even while facilities are being shut down (see *Nature* 342, 5; 1989). Some observers think that clean-up costs are likely to go higher still, so high that it may not be politically feasible to pay them.

Even if Congress does allocate the necessary funding, the current cost projections and the operation itself are predicated on the assumption that the Department of Energy will successfully complete its nuclear waste repositories in Nevada and New Mexico. But both of these facilities are far from completion and continue to be fraught with political opposition and technical problems.

Seth Shulman

Imaginary orderliness

SIR—The situation at the Natural History Museum (NHM) is as serious as any that has faced Britain's 'national' museums in recent times. Yet the bulk of Sir Walter Bodmer's Commentary (*Nature* **345**, 569; 1990) appears to ignore reality in a push for the status quo.

Professor John Evans, immediate past president of the Geologists Association, through his comments (reported by Henry Gee in *Nature* **345**, 99; 1990) on the corporate management proposals of the museum's director, Neil Chalmers, is working to advantage only the agenda of those intent on pursuing the narrow rational economic agenda. Statements such as "[the plan] has . . . been cobbled together in American-style business jargon presumably courtesy of Disneyland where many of the museum's administration went to learn such jargon" can surely be described only as ignorant nonsense.

The NHM is studying Disneyland so as to give visitors better service: that is good. Disneyland is expert at excellent customer services. That contributes more to the continuation of the NHM's outstanding work than abuse from single-interest groups who wish to brand everything about management as a fascist plot.

Evans' successor, Professor Beverly Halstead, long-time critic of changes at the museum in exhibitions which are based on very good research and sound judgement, has also done little to help the museum by his recent comments.

Detailed analysis of the corporate plan and of whether it is an appropriate response to the future is well worthwhile. But that exercise should surely use the same caution and logic as is applied by scientists to their own research.

The problem is the British government and its continued refusal to recognize the enormous importance of museums in *all* areas of science and education. And its failure to recognize their importance in economic terms is shown by recently published studies (for example, *The Economic Importance of the Arts* by John Myerscough, Policy Studies Institute, July 1988).

To help the museum and its staff and (most of all the public), the last thing one should do is to campaign on single-issue agendas such as taxonomy and the 'undesirability' of changing direction. I am astonished at the narrowness of view of many of the scientific groups expressing concern. Reading some of the draft letters that are circulating, one would imagine that the only problem resulting from reductions in the museum's funding was the loss of knowledge of sponges or crustaceans. Of course this is important, but it is the existence of the museum which is at risk. Only if that is secured will it be possible to discuss its shape and future programmes.

What would the Geologists Association do to reverse the trend at the museum towards salaries exceeding the total money granted by government? Presumably 132 years have taught them something. Try joining together with others concerned about the future of museums to put pressure on the government. It is a political issue after all.

DES GRIFFIN
(Chairman, Council of Australian Museum Directors)

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Australia

SIR—Sir Walter Bodmer, writing as chairman of the trustees of the Natural History Museum (NHM), has defended the recent corporate plan of this museum[1]. He argues that, given a shortfall in funds, the scientific programme was the only area left in which cuts could be made. He points out that many scientific posts have been lost in the museum over the past five to seven years by random natural wastage and claims that, because of this randomness, "the director and his senior staff [have had to] assess research priorities" so that any further cuts would occur in a more orderly way.

Such orderliness is imaginary, however, for the imposed cuts do not fit any scientific plan. They were decided by heads of departments in obedience to the command: "Your department will lose *x* posts and you will decide which ones".

This command produced many anomalies where the redundancies run counter to the ostensible aims of the plan. To give one example out of many[2], the museum, under the plan, is supposed to be more environmentally aware than in the past — and we accept this aim. Nevertheless, research is to stop in diatoms, which are first-class environmental indicators of particular value in reconstructing the course of global warming. Many of these anomalies have been mentioned in parliament by Mr Tam Dalyell MP[3].

The brutality of the plan, the high-handedness with which it was announced, and other insults dealt to us by management, have demoralized the staff and produced many requests for voluntary retirement additional to those which the management originally called for. It is true that these requests are randomly distributed scientifically, but so are the cuts proposed by the plan. Allowing some requested retirements would provide several free posts and enough money to restore some of the axed curatorial and research positions. We therefore urge management to reassess the cuts with all this in mind.

After all, Sir Walter Bodmer would never claim that the museum is stronger without several axed scientists who dearly wish to stay, including a certain world-famous archaeozoologist and an internationally known palaeobotanist.

P. E. J. WHEATCROFT
(Branch Chair)
R. P. S. JEFFERIES
(Secretary, Science Defence Committee)
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1. Bodmer, W. *Nature* **345**, 569–570 (1990).
2. Science Defence Committee *Corporately Planned Damage to Science* (IPMS (Natural History Museum Branch), 1990).
3. Dalyell, T. *Hansard 174 (118, for 11 June 1990), pp.18, 77, 102.*

Journal titles

SIR—I commend Dr Judah Rosner's comments on the unseemly dogmatism of the AST (assertive sentence title). Perhaps he is right in suggesting that some scientists embrace this style in order to impress granting agencies. But there is one other factor. In 1981, Martin Glennie and I submitted to *Nature* an article with the fuddy-duddy title "The use of univalent antibodies to kill tumour cells". It was published as the resounding "Univalent antibodies kill tumour cells *in vitro* and *in vivo*". Had we in the intervening months become more thrusting and positive? Not in the least. The title was altered in the editorial office of *Nature*.

G. T. STEVENSON

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Eminent engineers

SIR—Not only was President Herbert Hoover an engineer (*Nature* **345**, 106; 1990), but he had strongly held views on the value of education. He wrote in 1941 that "British universities [had] refused to incorporate engineering into their curricula until much later than the Americans. In the meantime, the American engineers — especially the mining engineers — flooded the British Empire" (Foreword to T. T. Read's *The Development of Mineral Industry Education in the United States*, New York, 1941).

We would certainly be helped if Hoover's positive attitude to training engineers prevailed in British politics today.

HUGH TORRENS

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Opportunities for the East

SIR—Further to the News article "Opportunities for the East" (*Nature* **345**, 196; 1990), readers may be interested to know that the International Agency for Research on Cancer (WHO), based in Lyon, France, runs a fellowship programme for research training in cancer addressed to young postdoctoral scientists. This programme was initiated in 1966 and has already provided over 70 fellowships to Eastern European scientists, thus contributing to the maintenance of contact with the international scientific community and to the development of cancer research in the home country of the Fellows.

R. MONTESANO

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Hungarian freedom

SIR—Steven Dickman writes (*Nature* **344**, 615; 1990) that "The old Hungarian University became a Romanian one in 1920, when Transylvania was given to the Romanians as part of the peace of Trianon. But the Romanian faculty was forced to flee to Sibiu in 1940 when the Axis powers gave the area back to Hungary". He omits to say that the original Hungarian faculty was forced to 'flee' to Szeged in 1920. In 1940, the original Hungarian University of the city (Kolozsvar in Hungarian, Cluj in Romanian) was permitted to come back. After the Second World War, according to the agreement overseen by the victorious four powers of Britain, France, the Soviet Union and the United States, it was stipulated that Romanian and Hungarian universities should coexist. "Until 1959 Romanian and Hungarian universities existed side by side, then they merged into a predominantly Romanian entity", according to Dickman. This is not exactly what has happened. The truth is that the Hungarian faculty was suppressed on orders from Bucharest. An eminent professor at the Hungarian university committed suicide and in his farewell letter wrote that his act was a protest against the violation of liberty and the decree of suppression.

When the university was founded in the last quarter of the nineteenth century, more than 80 per cent of the inhabitants of the city were Hungarian. This is evident from the tombstones in the city's one cemetery. More than 80 per cent of those buried before 1920 were Hungarian. Many great Hungarian writers and musicians (Bartok and Ligeti, for example) were born in Transylvania.

We should also remember that the principle of the Austrian Empire was "*Divide et impera*" ("Divide and rule"). The Austrian emperor always listened favourably to the different ethnic groups of the empire.

Dickman states that there are now "30 per cent ethnic Hungarians, most of them housed in the huge blocks of flats characteristic of the Ceasescu era". He does not say that the Hungarians were expelled from their lodgings, which were then destroyed by bulldozers, and their former inhabitants were forced to live in "the huge ugly blocks".

Ceausescu was a terrorist who practised genocide and had a terrible hatred of Hungarians. It is this type of sentiment that caused the terrible tragedy in Tirgu Mures: the killing of unarmed Hungarian civilians, the wounding of more than 300 Hungarian civilians, the putting out of the eye of Suto, the well-known Hungarian writer, and other atrocities. Nothing similar occurred during more than 1,000 years of Hungarian rule.

The Hungarians of Transylvania are demanding only to be able to live freely without oppression and in harmony with the other ethnic groups.

ZOLTAN OVARY

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More on the Shroud

SIR—Regarding the procedure for obtaining samples from the Shroud of Turin for radiocarbon testing, M. S. Tite of the British Museum (*Nature* **332**, 482; 7 April 1988) says, "all stages will be fully documented by video film and photography". However, the official report of the test results (*Nature* **337**, 612; 1989) says: "All these operations, *except* for the wrapping of the samples in foil and their placing in containers, were fully documented by video film and photography" (my italics).

The key word here is "except"; its presence raises serious questions. Did the custodians of the Shroud agree that "all stages" of the procedure would "be fully documented" as Tite indicates in his letter of 7 April? If so, why did they make and suddenly break that reasonable agreement? (Note: The sampling of the Shroud took place on 21 April — just two weeks after Tite's reassuring letter appeared in *Nature*.) Why wasn't the entire procedure documented on video film and made available to the public? Without full documentation how can one be certain that it was the Shroud — and not another cloth — that was carbon dated? At what point in the sampling and sealing procedure did filming end — and why was it ended?

As Tite was the independent overseer of the carbon dating test and the guarantor of its unequivocal reliability, he has an obligation to address these unanswered questions. (That obligation would be very apparent had the Shroud been carbon dated to the first century.) And it should be noted that the man of the Shroud and the person(s) who imprinted his Christlike image are, as yet, unidentified — and not everyone is convinced that the Shroud is mediaeval as the results of carbon testing indicate.

ROBERT HALISEY

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TITE REPLIES—I confirm that, as stated in the *Nature* article, the wrapping of the samples in foil and their placing in containers was not documented by video. This was because we were continuing to follow the blind testing procedures according to which only the Cardinal, Professor Gonella and myself were to know which containers held the Shroud samples.

This aspect of the procedure was, I admit, somewhat illogical, as by this time we were aware that, because of the unusual weave of the Shroud, blind testing was not feasible without unravelling the samples. However, I should emphasize that it was the Cardinal and myself who were guarantors of the samples and that the video film was intended as an *aide-mémoire* rather than being meant to provide definitive proof of the identity of the samples.

M. S. TITE

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HUGO's finances

SIR—We are, of course, delighted that the Howard Hughes Medical Institute (HHMI) has made a grant to HUGO of a substantial sum — $1 million over four years. However, your report (*Nature* **345**, 100; 1990) that this was HUGO's "first funding of note" is simply not true and we would like to set the record straight.

Earlier this year, you yourselves noted (*Nature* **334**, 5; 1990) that the Wellcome Trust (not Burroughs Wellcome) had made a three-year grant to HUGO, to cover the costs of its London office and its European activities. The Wellcome Trust's award is for approximately £175,000 in the first year (with another £50,000 available for programme activities yet to be defined) and will, overall, be of about the same order of magnitude as the HHMI grant.

WALTER BODMER
(President)

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Global leadership in semiconductors

Hajime Karatsu

Success in the semiconductor industry requires bold investment in new technology and total commitment to quality. US companies will never catch up with their Japanese counterparts until they learn these and other lessons.

WHAT is the secret of Japan's success in the semiconductor industry? In the early 1970s, the world market for semiconductor products was dominated by the United States. But by the 1980s Japan had taken over the key sectors of the market (see figure over) and now, for example, supplies about 80 per cent of the world's memory chips. If the United States is to compete once again, fundamental changes in the attitude of industry are needed, from the top levels of management down to the shop floor.

Technological progress

The key to the next generation semiconductor market is investment bold enough to keep up with the pace of technological progress. The success of Toshiba, which has won a large share of the market for 1-megabit DRAM (dynamic random access memory) chips, demonstrates how investing with vision can put a company ahead of the competition. Toshiba started heavy investment in its semiconductor division in 1984 and managed to supply the 1-megabit DRAM chips two years before its competitors. With this foothold, the company could sell at a profit while taking rapid depreciation on the advanced equipment needed for production, and thus was better positioned than the late starters. As the result of this foresight, Toshiba's semiconductor business reportedly accounted for half of its profits of $1.6 thousand million in 1989, even though the company is a highly diversified electronics giant with products ranging from locomotives to washing machines.

Today's fierce competition means that tomorrow's technology is being developed with ever-increasing speed. Adapting to this lightning pace requires aiming investment at the advances of the future. Toshiba's investment strategy was at least one year ahead of that of its competitors.

Shortening the time taken to develop new products is also critical. In the semiconductor industry, technological improvements are made in rapid succession, and introduced just as quickly to the shop floor. The old technology cannot match the new in cost and performance. Failure to adopt new technology will mean lost profits further down the road, and the longer the delay the greater the loss.

This may be why semiconductor firms in the United States have lost competitive ground to their foreign counterparts. A short-sighted focus on quarterly profits runs counter to the nature of the semiconductor business.

Commitment to quality

The term 'learning curve' is losing its significance in the semiconductor industry as a result of refinements in manufacturing technology during the past decade: we are now able to derive high yields from new chips in the first period of production.

One reason for this progress is the concept of 'super clean' technology, developed in Japan at Tohoku University. The concept is simple: it involves striving for perfect cleanliness at every stage of the production process. Gas and materials are pure; machinery, pipes, wells and lighting do not generate dust; and workers are free of contamination. Production yields have improved dramatically where this approach has been adopted.

Almost all materials and equipment used today, however, fall short of these standards. In the 5 February issue of *Business Week*, an article entitled "The Future of Silicon Valley" contained a photograph showing a cloakroom in a semiconductor factory and careless handling of workers' clothes. In Japan, workers' clothes are washed every time they are used. When I was at Matsushita Communication Industrial Co., workers making engine control units for automobiles were required to change their gloves every three hours to prevent dust from entering the most high-precision stage of the process. Thanks partly to this precaution, the defect rate was less than 0.1 per cent.

Manufacturing is by its nature a continuing battle against error and uncertainty. This is doubly true for the semiconductor business. Procured materials might not fit the specification exactly, skilled operators might get sick, machines can perform unevenly, designs may have to be changed slightly at the request of customers, and a microscope might reveal bacteria on a chip.

Some engineers tend to think about manufacturing flaws in terms of black or white, but the 'grey areas' can be important. Forcing distinctions may mean that the root of the problem is never reached.

At Matsushita, we received some defective products from a factory in Singapore that was affiliated to a US company. In one package, we found that contamination on the chips had corroded the aluminium pattern. When the factory manager visited Japan, I asked him to be more careful about his workers' clothing and masks and to review procedures for protecting against contamination. He replied that his men applied passivation covers to protect the chips from dust, so there must have been some other cause for the defect. The factory manager would not budge from his viewpoint. His attitude could be paraphrased as follows: "We tried as hard as possible. Why are the Japanese complaining about trifles?". I wonder what the response would have been if such defects had been found in a Japanese factory. Because dust is intrinsically harmful to semiconductors, all employees would have redoubled their efforts to prevent a recurrence, even if passivation covers had been applied.

Reaching a decision without exploring the middle ground, as the factory manager did, is not conducive to solving problems. This syndrome is exacerbated by confining employees to their specialized fields, where they are likely to develop a compartmentalized, self-serving attitude that may inhibit them from admitting their own mistakes. Attitudes such as that of the factory manager will not help to improve production efficiency and/or quality. Even if errors can be explained away, the product has the final word.

There are three ways to address the grey areas of manufacturing flaws. The first is to improve robustness against variation or error in production by selecting optimum parameters for materials, components and machine settings during design engineering. A new design methodology that has recently attracted attention is the Taguchi method, developed by Dr Genichi Taguchi of Bell Laboratories, for designing memory chips. It is gradually gaining favour in the United States and Japan. The second is to make the grey areas as small as possible by using 'super clean' technology. The third is aggressively to seek out sources of manufacturing errors that cannot be totally eliminated — that is, total quality control.

'Bottom up' strategy

Companies in Japan and the United States tend to have fundamentally different approaches to commercializing new technology. To borrow a metaphor from America's favourite pastime, US firms try to hit home runs, whereas Japanese companies try for single and squeeze plays. When carbon fibre was developed, for example, US engineers applied the technology to aviation, envisaging halving aircraft weight. But a new technology such as this tends to be accompanied by defects, requiring a long time for quality to be assured. So Japanese companies started using carbon fibre for making lightweight golf-club shafts and fishing rods, products in which defects were more easily corrected. Because of the mass market for these products, manufacturing volume increased rapidly, quality improved and costs declined. With this experience behind them, companies began applying carbon-fibre technology to aircraft. Today, the Japanese control 60 per cent of the global carbon-fibre market. I call this approach the 'bottom up' strategy.

The difference in approach to technology commercialization is also evident in LSIs (large-scale integrated circuits). Companies in the United States began by using them in sophisticated applications, such as airborne equipment, missiles and computers. Japanese companies, on the other hand, first used the chips in small calculators, leading to the development of pocket models. At the time, the cost of LSIs was too high for the consumer market, but Japanese engineers correctly perceived that it would decrease quickly as a result of widespread demand for more-compact calculators. When the models were first manufactured, an LSI chip cost $70; today, it costs only $1.

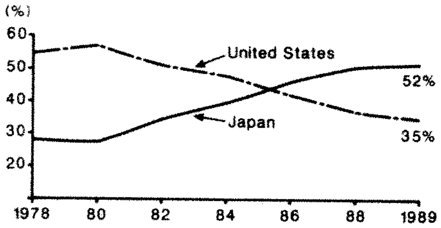
A critical factor in the development of pocket-sized calculator chips was CMOS (complementary metal-oxide semiconductor) technology. Japanese companies concentrated on this technology to lower the power consumption of calculators and wristwatches. This was the key to the development of random-access memory chips.

Another factor supporting Japanese companies' competitiveness involves the US–Japan semiconductor agreement, instigated by the United States. The anti-dumping provisions in the agreement drove market prices to double or triple. As Japanese semiconductor manufacturers also make computers, domestic appliances and electronic components, their electronics products were protected from price rises. Because intra-company shipments are not covered by the agreement, the parties harmed most by the sanctions were the US companies that depended on outside chip supplies. In effect, it was the goose that laid the golden egg, bringing in an enormous amount of money, which was in turn reinvested to further advanced semiconductor technology.

Defence market

Compared with the fierce consumer market, the defence market is rather sedate. At first sight, it would seem to be attractive because it demands the most advanced technology with less regard to cost, and the government guarantees the supplier's profit. When semiconductor technology was being developed, support from the US Department of Defense was important in making possible large investment for research and development. Some key technologies were developed as a result of support for defence projects.

But these merits conceal a pitfall for the semiconductor industry. Defence applications often do not keep up with the fast pace of technological progress. Once a chip is installed in a specific weapons system, the providers are requested to supply it for at least the next ten years. Any modification or improvement is not allowed in the name of standardization.



US and Japanese shares of the world semiconductor market (on the basis of sales).

In the civilian market, by contrast, companies jockey for competitive position by continually introducing improved technologies. Inferior products are forced out of the market. In Japan in 1989, 115 new facsimile models and 96 new copier models were released, attesting to the effects of stiff competition in the consumer and business markets.

It should also be borne in mind that production volume for consumer use is always greater than for defence use. Take, for example, the laser diode, a key component of compact-disc players. Hundreds of thousands of these diodes are manufactured every month in Japan. Technology developed for the mass market often has defence applications: CCDs (charge-coupled devices) for camcorders — produced in the same volume as laser diodes — are also used in the 'smart bomb', where they act as guides to the target.

In Japan, a stream of sophisticated new technologies superior to those created for defence use have been developed under the stimulus of free competition. The use of such advanced components is expanding the consumer market. In Japan, an auto-pilot model airplane equipped with a microprocessor and gyroscope costs about $300 in hobby shops, and robot toys are very popular with young people.

High-definition television (HDTV) is one of the hottest technologies of the next generation, even though work on it started more than 15 years ago at the research institute of the Japan Broadcasting Corporation. Since last year, one hour of test HDTV is broadcast every day by satellite. Meanwhile, Japanese manufacturers of domestic appliances are investing large sums to develop both new chips and display technology, including liquid-crystal flat displays (LCD). Japanese electronics companies began developing giant-size flat-screen display technology, for which their expenditure for constructing LCD factories is estimated at $10 thousand million last year. Wall-type TV screens should be on sale in Japan within five years. HDTV technology also has important defence applications, and is sure to be transferred to the military field — Japan has already supplied HDTV equipment to the United States for possible use in the strategic command room.

Attracting talent

In 1987, I attended a conference in Finland at which a vice-president of McDonnell Douglas lectured on the next-generation transport aircraft. Through the introduction of such technologies as a nonducted-fan jet engine and a low-friction wing, he said, the aircraft will consume only about one-third the fuel of today's transports. These projects are sponsored by NASA.

As I listened to him speak, I imagined myself as a graduate of a US engineering school and asked myself if, with such exciting innovations in the aircraft industry, I would choose to work for the automobile industry, which is battered by overseas competition? I would opt for the aircraft industry, of course, and that is a cause for concern, because any industry that cannot attract talented engineers is heading for decline.

During a break, I asked the McDonnell Douglas executive about the size of the aircraft industry. He replied that it had $80 thousand million in annual sales. Sales in the US automobile industry total $270 thousand million. Given the strategic economic importance of the automobile industry, it has to attract the best engineers. The iron and steel industry lost much of its competitiveness because management, protected by quota systems, failed to adopt new technology. It thus became an undesirable sector for young engineers. The size of the steel industry is about $30 thousand million, just half that of the aircraft industry. The economic consequences would be much more severe if the automobile industry suffered the same fate.

I cannot stress too much the need to make those industries fundamental to a nation's economy attractive to young graduates. In Japan, we have been fortunate to have been successful in drawing many of the brightest graduates to work in them. But all industrial nations could do better in that respect, not least in the United States. ☐

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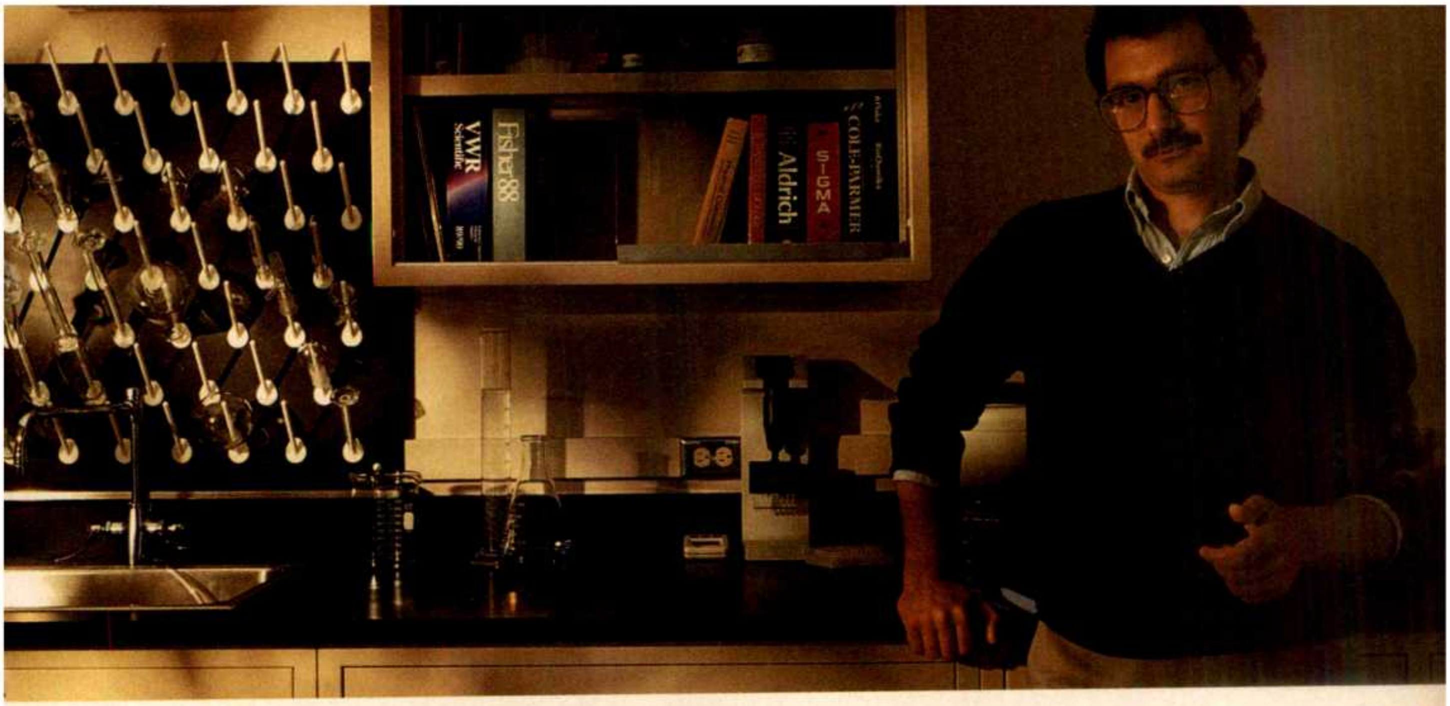

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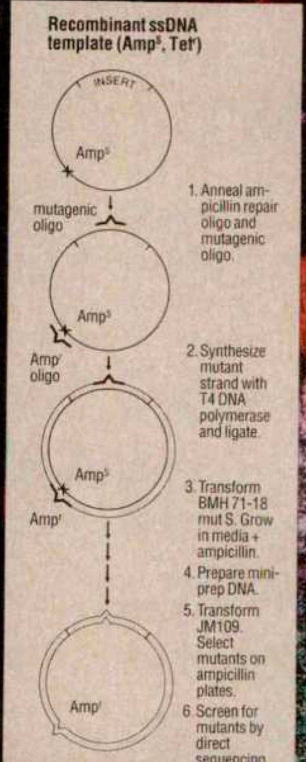


Recombinant ssDNA template (Amp^s, Tet^r)
INSERT
Amp^s
mutagenic oligo
1. Anneal ampicillin repair oligo and mutagenic oligo.
Amp^s
Amp^r oligo
2. Synthesize mutant strand with T4 DNA polymerase and ligate.
Amp^s
Amp^r
3. Transform BMH 71-18 mut S. Grow in media + ampicillin.
4. Prepare miniprep DNA.
5. Transform JM109. Select mutants on ampicillin plates.
Amp^r
6. Screen for mutants by direct sequencing.

Stefan Marinov's seasonal puzzle

With yet another holiday season brewing up in the Northern Hemispehere, readers may wish to brood on a conundrum devised by an anti-relativist. No prizes are offered for a solution.

STEFAN Marinov, the exiled Bulgarian physicist now living in Graz in Austria, is himself a puzzle. He is indefatigable in the prosecution of what seems to be his only cause, which is to prove that Einstein's theory of relativity is a pack of lies.

Marinov claims, among other things, to have shown by direct measurement that the velocity of light differs according to the direction of its travel along a fixed path. He has also partly developed what he says is a perpetual motion machine, a kind of Wimshurst machine driven by an electric motor which, when last inspected at this journal's London office, was said to require a car battery for its operation.

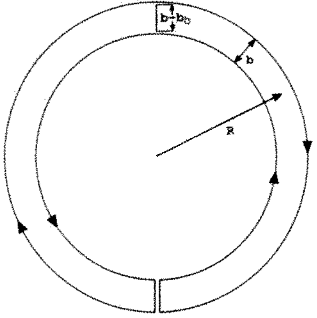
But most of Marinov's work is theoretical. Over the years, he has bombarded this and other journals with a series of papers with titles such as "The myths in physics" and "Violations of the laws of conservation of angular momentum and energy". Details of his correspondence with several editors may be found in his series of volumes called *The Thorny Way of Truth*, of which seven volumes have now been published by International Publishers (East–West) at Graz, of which Marinov appears to be the sole owner.

(From time to time, Marinov threatens to immolate himself in front of a British embassy or consulate if he cannot get an answer to a question about the publication of one of his papers. Some years ago, when embarked on such an enterprise outside the British consulate at Genoa, Marinov ran away when the police approached. Afterwards he explained that he had done so because he was in Italy illegally, without a visa. Mercifully, he has not threatened to immolate himself on *Nature*'s account for many months.)

Part of everybody's difficulty is that too little is said about the details of the experiments whose results are said to contradict common expectation for even sympathetic critics to be able to provide constructive statements of how they and Marinov part company. Another is that Marinov's single-minded zeal for his own conviction considerably outstrips the zeal of those who may disagree. But from time to time, in Marinov's copious writings, there are relatively simple arguments that appear accessible even to those still at high school. Here is one series of *gedanken* experiments presented as if it were a Christmas puzzle (the original intention), with some helpful (or misleading) hints for its solution.

The figure shows a pair of circular conductors arranged as two concentric circles. Equal electrical currents are circulated in each, but in opposite directions. The simplest way of creating this arrangement is to cut through the concentric pair at some point and to join the loose ends in pairs by short lengths of straight conductor. An electromotive force applied anywhere along the conductor will engender a current which must be everywhere uniform. At the bridged gap, there will be equal currents flowing in opposite directions, so their influence on the magnetic fields in the concentric gap will be zero.

The device is thus a means of arranging that there is a uniform magnetic field in the space between the concentric circles in a direction perpendicular to their plane (downwards into the plane of the paper when the current in the circuit flows in the direction indicated). The sensor in the experiment is a conductor long enough just to bridge the gap between the concentric circles and mounted on thin insulating supports in such a way that it can be made to slide around the circle. The objective is to measure the voltage across the sliding conductor, either by a standard voltmeter or by a condenser whose accumulated charge will be a measure of the voltage in a steady state.



The simplest case is when the sliding conductor is at rest. Then there is no voltage. Right? Next comes the case in which the sliding conductor moves at uniform speed around the concentric gap, always pointing along a radius of the concentric circles. As the slider moves, it will cut through magnetic lines of force at a constant rate, so there will be a constant voltage across the ends. The polarity of the slider will depend only on the direction of the current in the concentric circuit, and not on whether the slider moves clockwise or anticlockwise. Right again?

Now comes the tricky part, at least so far as Marinov is concerned. What happens if the sliding conductor is fixed in space, but the underlying concentric circuit is rotated about its centre? Relativity theory naturally predicts that the voltage across the sliding conductor would be the same as in the first experiment, and with the same polarity. On the other hand, questions may be raised about the degree to which the pattern of magnetic forces generated by the current is dragged around the ring by its rotation. Maybe there is a smaller voltage, but with the same polarity. What, asks Marinov, is the answer?

The second conundrum is superficially simpler: simply rotate the apparatus in its own plane, about the centre of the concentric circles. (There will be a small voltage due to the Earth's magnetic field, but this may safely be neglected.) Is there now a voltage, and with what polarity? If the answer to the first question is "Yes" the answer to the second must be "No", and vice versa. Readers are invited to make up their minds before reading on.

Marinov's own answers are unambiguous. Vice versa wins the day. When the underlying concentric circuit is rotated and the slider is kept fixed, there is no voltage across the movable conductor. But when the whole apparatus is rotated about its centre, the voltage across the now-moving sliding conductor is identical with that obtained when the slider is moving relative to the concentric circuit.

The implications are evidently important. The null answer to Marinov's first question implies that relativity has vanished through the window, the affirmative answer to the second implies that an isolated apparatus carrying a circulating current will generate a voltage when rotated, which raises forbidden questions about absolute space. Indeed, Marinov has devised a *gedanken* measurement of the Earth's velocity through space by stretching his concentric circuit into a linear version of it. He also claims that these violations of simple expectation are the basis on which his perpetual motion machine is built.

What is the truth? Nobody is quite sure, for nobody has done the experiments — not even Marinov. Indeed, one's confidence in the whole enterprise is somewhat undermined by Marinov's flat statement that he does not bother to repeat experiments whose outcome must be obvious. For one whose confidence in his own heterodoxy appears to be sustained only by his confidence that experiments will prove him right, Marinov seems curiously passive in the business. **John Maddox**

ECOLOGY

A chaotic synthesis

Rory Howlett

ECOLOGY has now emerged as an exact science[1]. This was the message of a recent meeting on the regulation and relative abundance of plant and animal populations*, an area where synthesis is not only possible, but probable (R. Southwood, Oxford University).

A growing trend in ecology is the analysis of long-term datasets to study the dynamics of populations either in isolation or when interacting with other species or variable environments. A common observation is that the apparent variability of population densities increases when calculated over longer periods of time[2]. Whereas plant populations have rather tame, often successional dynamics with recruitment depending upon the death of dominant plants (M. Crawley, Imperial College at Silwood Park), animal populations exhibit a vast array of dynamic behaviours, including periodic cycles and apparent chaos (I. Hanski, Helsinki University). Much attention is paid to the factors underlying population regulation, defined as long-term persistence and fluctuations within limits, the lower limit being greater than zero (see Murdoch and Walde in ref. 1). Comparisons within and between taxa show that species tend to have more constant populations when there is some dependence of *per capita* growth rate on population densities. But such density-dependence, although apparent in some long-term time-series, is generally difficult to detect, even when it is assumed to be operative. Moreover, as Hanski points out, local populations tend to be embedded in more broad-scale 'metapopulations' — dynamic entities in which local populations can become extinct only to be re-established later by dispersal — and this will critically affect their local dynamics.

The difficulties involved in detecting density-dependent regulation of animal populations were highlighted by Anne Keymer (Oxford University), whose group has been grappling with the ecology of human helminth infections. These parasites are generally highly aggregated among hosts and the overall populations tend to be temporally stable. Furthermore, interventions designed to control disease have resulted in large-scale population perturbations from which recovery is often rapid, suggesting strong regulation. But the actual density-dependent mechanisms are hard to pin down. They may arise from competition among parasites within hosts when at high density, or from acquired immunity of hosts after long exposure to parasites. Keymer argued that the latter mechanism appears, on present evidence, to be the more important.

Populations and communities are often very complex, but the very nature of this complexity can sometimes provide important insights into regulatory processes. As pointed out by Peter Chesson (Ohio State University), populations have internal structures that stabilize them and favour the co-existence of competing species. Spatial heterogeneity in the environment also affects population dynamics profoundly. This theme was expanded by Peter Kareiva (University of Washington), whose previous work on the dynamics of ladybirds and their aphid prey in fields of goldenrod has shown that the dispersal behaviour and demography of interacting species play crucial roles in determining the response of populations to mosaic environments (see ref. 3). In the case of host-parasitoid interactions, patchiness in the environment can be stabilizing (M. Hassell, Imperial College at Silwood Park; S. Pacala, University of Connecticut). Theory suggests that this effect comes from variation in the amount of parasitism from patch to patch. Parasitoid attack rates can vary between patches in ways that may or may not depend on host density, but the dynamical consequences of this variation are difficult to assess. But recent work shows[4] that the stability of host-parasitoid systems can be predicted using the simple rule: 'overall population densities will remain roughly steady from generation to generation if the coefficient of variation squared (CV^2) of the density of searching parasitoids in the vicinity of each host exceeds approximately unity'. When this criterion is applied to the real datasets and partitioned into density-dependent and -independent components, it suggests that, for 9 of 34 published studies, heterogeneity is large enough to stabilize dynamics and that density-independent heterogeneity is often the main stabilizing factor.

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Tropical deforestation — ecologists are in a battle against time to understand the dynamics and diversity of the world's most complex and intractable ecosystems.

One of the most exciting areas of population ecology is the application of techniques developed to investigate the dynamical behaviour of complex systems (H.C.J. Godfray, Imperial College at Silwood Park; S.P. Blythe, University of Strathclyde). Recently, ideas have been developed for distinguishing measurement error from chaos in ecological time-series (G. Sugihara, Scripps Institution of Oceanography; see ref. 5); these have been successfully applied to data on measles, chickenpox and marine phytoplankton populations. But complexities arise when time series are subject to secular trends or darwinian evolution — an important consideration when analysing data such as those available for fish populations under heavy and sustained fishing pressure (J. Sheppard and D.H. Cushing, MAFF Directorate of Fisheries, Lowestoft). Indeed, the interplay of population dynamics and the evolutionary process is expected to influence population persistence (J. Travis, Florida State University), and even trends in biological diversity over geological time (J. Valentine, University of California, Santa Barbara).

Of all ecosystems, tropical forests must rank among the most complex. The challenge of understanding them has been taken up by S. Hubbell (Princeton University) and his colleagues, who have collected vast amounts of data on spatial patterns of sapling recruitment in a 50-hectare plot of a neotropical forest on Barro Colorado Island, Panama. They have used these data to study how tree species diversity is maintained. Strong negative conspecific effects on sapling recruitment in some of the densest species may prevent them from completely dominating the canopy, but many species do not show these effects, and Hubbell concludes that they may play only a limited role in determining the dynamics of a forest ecosystem. Studies of similar plots of forest located at sites right around the tropics, Hubbell hopes, will allow the development of a 'statistical mechanics' of complex communities.

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* *Regulation and relative abundance of plant and animal populations*, The Royal Society, London, 23–24 May 1990.

Despite these advances, great gaps remain in our knowledge of even basic ecological facts, such as the total number of species on the planet (R.M. May, University of Oxford and Imperial College, London). Only through understanding the structure and regulation of communities can we hope to plan the conservation of life's diversity. ☐

Rory Howlett is an Assistant Editor of Nature.

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OPTICAL ASTRONOMY

Are spiral galaxies opaque?

Michael Disney

ASTRONOMY is bedevilled by the presence of clouds of obscuring smoke mixed with the gas that drifts between the stars. This smoke (or dust, as it is more widely, but incorrectly, called) cuts off from sight much of the Universe and makes it difficult for us to interpret what we do see. On page 153 of this issue[1], Valentijn presents evidence that spiral galaxies are not, as has long been supposed, transparent, but are heavily clogged up with their own interstellar smoke. If he is right, rethinking of some major issues will be required.

If, as Valentijn suggests, we are seeing only the outer crusts of galaxies, we cannot so glibly assume that exotic 'dark matter' is necessary to explain the rotation curves of spiral galaxies (that is, their rotation seems to imply that more mass is present than is inferred from their optical radiation). At least in part, the hidden matter may instead simply be stars buried beneath the smoke. And the 'hidden starbursts' that have been invoked[2] to explain the surprisingly large infrared luminosities of spiral galaxies seen with the IRAS satellite may not be so widespread — the infrared could be merely starlight from obscured stars, reradiated by the cold smoke. We will also have to reassess the true proportion of galaxies undergoing nuclear activity, currently thought to be one or two per cent, as we may be seeing only the least obscured nuclei. If, furthermore, galaxies really are opaque, then as they pile up in the distance and fill our field of view, they will block out the more distant Universe at optical and ultraviolet wavelengths, cutting off high-redshift quasars and spoiling our view of galaxy formation.

The smoke in our own Galaxy strongly affects what we can see of it. On a moonless night the dark-adapted eye can see dark clouds of smoke outlined against the Milky Way. Optical radiation from the Galactic nucleus is attenuated by no less than 18 orders of magnitude. Fortunately, however, we live in the outer, relatively unobscured periphery of our Galaxy, so that if we look away from the plane of the Milky Way we get a fairly unobscured view of the extragalactic Universe. Astronomers in the majority of solar systems would not be so lucky, being forced to rely on longer-wavelength infrared and radio radiation to which smoke is more transparent.

When we observe another galaxy we have somehow to allow not only for obscuration along our line of sight but also the internal extinction within that galaxy itself due to its own smoke. How might this be done? In a highly influential paper in 1958, Erik Holmberg[3] suggested that we compare the properties of spiral galaxies tipped at different angles to our lines of sight. If they are perfectly transparent, their luminosities will be the same in all directions. But if they are perfectly opaque, they will look brighter when seen face-on, thus subtending the largest area of sky. Holmberg devised and carried out the classical test for opacity in spiral galaxies by comparing their apparent luminosities (or surface brightness) with their inclinations to the line of sight. From that test he and his successors concluded that spiral galaxies are largely transparent: when seen face-on, less than 40 per cent of their light is extinguished by internal obscuration[4].

Although simple in principle, Holmberg's test is very difficult to carry out in practice. How can one be certain, for example, that the edge-on galaxies in one's sample are drawn from the same intrinsic population as the face-on galaxies? One may not be comparing like with like. And even if such selection effects can be discounted, one cannot infer the extinctions from the observations without first adopting some more or less arbitrary form for the distribution of the smoke. If sunk in the galactic plane or gathered into compact clouds, it will obscure less than if it were widely dispersed. In any case, optical observations suffer from a systematic bias: more light will come from the least obscured parts of the galaxy, leading to a systematic underestimate of its extinction[5]. Most astronomers over the past 30 years have either ignored or discounted these difficulties and accepted Holmberg's view that spirals are largely transparent.

Such were the difficulties of observation in his day that Holmberg was forced to work with a sample of less than 200 galaxies. Taking advantage of the new automated and digitized microphotometers, Laubers and Valentijn[6] have measured at least 10,000 bright spirals appearing on the European Southern Observatory Survey plates. It is from this sample that Valentijn now draws his conclusions. With thousands rather than tens of galaxies to work with, he can be more selective in choosing his samples and, by comparing different samples, can make some esimate of his own selection effects. He compares the surface brightness of 2,500 spiral galaxies with their inclinations deduced from the ratio of their major to minor axes. Unlike Holmberg's, Valentijn's galaxies fit perfectly the totally opaque model, and are certainly inconsistent with the transparent or even semi-transparent models.

Valentijn's results clearly do not agree with Holmberg's or with Heidmann's[7]. Why this should be so is not clear. Valentijn believes that it is due partly to the greater selectivity that he can afford, and partly to the more precise definition and measurement of surface brightness afforded by his instrument. In one sense Valentijn's result is almost too good to be true, because it implies that even the outer parts of spiral galaxies are optically thick — a surprising result given the small amounts of gas detected there. To confirm the result requires mapping of spiral galaxies in the infrared, where they should be optically thin, and in the difficult sub-millimetre region, in which most of the cold ($\lesssim$ 20 K) dust should

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show up in emission. Valentijn's result is more likely to stir up than to end the controversy.

If spiral galaxies are opaque, astronomers, and cosmologists in particular, will need to revise some of their ideas. Indeed, Davies[8] and Valentijn[1] have both now pointed to the difficulty of naively inferring the existence of missing mass from optical photometry (although they by no means discount the possibility of dark halos), and Wright[9] has demonstrated recently that the decline in quasar numbers at high redshift can be plausibly accounted for by the presence of opaque galaxies in the foreground. ☐

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VISUAL NEUROSCIENCE

A causal chain in motion

Andrew Parker

EVER since microelectrodes reliably picked up action potentials from sensory nerve cells, there has been a continuing debate about the relationship between the firing patterns of cells and the perceptual consequences for the organism. In the early experiments, the emphasis was on simple detection of sensory stimulation[1–4]. This work produced some elegant and exciting conclusions. The retinal ganglion cells, whose axons communicate with the rest of the central nervous system, can detect flashes of light so weak that they contain only a few quanta of light[5,6]. The quality of sensation evoked by stimulation of single visual and tactile afferents may fall into discrete and readily identifiable perceptual classes[7,8]. As shown by Salzman, Britten and Newsome on page 174 of this issue[9], the same questions are being asked nowadays of regions of the brain that are more evidently concerned with higher-order perceptual analysis.

One suitable candidate region is the cortical visual area, V5 (or middle temporal, MT), whose function has been linked to the higher-order processing of visual motion[10–12]. Individual cells in this area are selectively excited by particular directions of visual motion. Last year, Newsome's group at Stanford showed that the sensory thresholds of single neurons in MT could match, or even exceed, the behavioural performance of the animal in a motion discrimination task[13]. Moreover, on occasions, there can be a reliable trial-by-trial association between the firing of single neurons and the behavioural response[14]. Now, with another clever shuffling of classical techniques, the same group[9] has studied the application to cortical neurons in MT of a weak, spatially localized, electrical stimulation at exactly the moment that a perceptual decision is required. Such a stimulation must slightly alter the firing patterns of MT cells and, as might be hoped, it can bias the perceptual decisions in a selective manner.

The experimental strategy relies on the functional organization of the properties of cells in MT. Not only is there a topographical map of the visual field onto this piece of cortex, but neurons with similar selectivities for the direction of motion are clustered together — probably in some form of columnar arrangement. Salzman *et al.* carefully determined the motion properties of a small region of MT by analysing the excitation of neurons in response to visual patterns of moving dots. Next, they presented a target of moving dots restricted to the same patch of the visual field over which the neurons could be excited. The dots could move either in the direction locally preferred by the neurons or in the opposite direction.

The animal had been trained previously to signal its judgement of perceived direction by shifting gaze from the initial fixation point to one of two locations either side of the target. In order to push the perceptual decision to threshold limits, only a fraction of dots in the target actually moved in the designated direction. The remainder moved in random directions and the measure of motion stimulus strength was the percentage of dots moving in a consistent direction. The critical result is that local electrical stimulation, applied at the same time as the motion target, may add to the nervous system's estimate of motion stimulus strength in a direction-specific manner. The authors present evidence that the electrical stimulation does not directly elicit the eye-movement response that is used as a monitor of the perceptual decision.

There are two surprising features of this work. The first is that electrical stimulation of a very small region of the cortex significantly affects a perceptual decision of the whole organism. Perhaps stimulation affects only a few cells at the stimulation site or perhaps it excites a particular neural circuit serving motion analysis, but the result does imply an extreme localization of function. Indeed, certain cortical locations in MT seem to be especially favourable for eliciting shifts in perceptual decisions.

On the other hand, the applied stimulation is rather nonspecific in its temporal aspects. Although it was confined to the duration of the visual stimulus, there was apparently no need to match accurately any naturally occurring temporal pattern of excitation for the cells. This gives little support to the notion that the exact temporal pattern of action potentials codes for the attributes of a stimulus. But this issue (and a number of related ones) can be resolved properly only when the experimental approach is modified to allow a more detailed analysis of the subjective qualities of sensation evoked by stimulation.

The information derived from this type of experiment should have at least one longer-term benefit. If the causal links between nervous activity and behaviour of the organism can be placed on a firmer basis, this knowledge will help to decide what kind of picture of brain activity is needed to assess neural function and dysfunction. The consequences for developments in brain-scanning techniques for use in clinical diagnosis could be profound.

It is an interesting comment on scientific fashion that 20–25 years ago the purist wing of artificial intelligence might have dismissed this kind of experiment as fatuous — after all, imagine trying to analyse the operation of a standard digital computer with a voltmeter, battery pack and a switch. Yet the brain clearly does not have a von Neumann architecture and has, through the course of evolution, generally adopted modular structures that have different functional properties, especially at a cortical level. Now the debate in parallel computing architectures is much concerned with structural organization and its consequences for computing efficiency, even at the hardware level. Although neural-network architectures are essentially parallel, their development has had separate motivations, often emphasizing the intrinsic value of distributed representations of information. How network systems can accommodate experimental results that imply highly localized and specific functional modules is somewhat problematic. ☐

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PALAEOCLIMATE

Ups and downs of African lakes

Neil Roberts

WHEREAS Quaternary climatic change in high latitudes is dominated by the buildup and decay of ice sheets, the dominant climatic signal in low-latitude regions is not glaciation but precipitation. In areas such as inter-tropical Africa, arid and humid phases have alternated as the monsoonal circulation system has been weakened and strengthened. Tropical palaeoclimate is recorded most clearly by changes in the water level and chemistry of 'non-outlet' lakes. These had low water levels, indicating an arid climate, during and after the last glacial maximum (21–12.5 kyr ago), followed by a period of high lake levels (a humid climate) during the early part of the present interglacial (12.5 – 5 kyr ago). Unlike the slow responses of glaciation and vegetation to high-latitude climatic change, many low- and mid-latitude lakes respond rapidly to hydroclimatic change. On page 141 of this issue[1], Gasse *et al.* present high-resolution stratigraphic records spanning the period from 15 to 7 kyr ago from two such 'responsive' lake basins, one on each side of the Sahara. These two records show that, like the eustatic sea-level record of Northern Hemisphere deglaciation[2], the last arid-to-humid transition in Africa was not smooth, being broken by climatic events lasting 100 – 1,000 years.

Major climate changes driven by the Milankovitch cycle of the Earth's orbital variations are well established. Sub-Milankovitch climatic oscillations (known traditionally as stadials and interstadials) superimposed on these longer-term astronomically induced variations have also long been recognized, but they are currently becoming of increased palaeoenvironmental interest[3].

Their causes remain a subject of debate, but these almost certainly include agencies such as major emissions of volcanic dust and the formation of North Atlantic deep water[3,4]. Of these palaeoclimatic oscillations, the so-called Younger Dryas event (11–10 kyr ago) has received the greatest attention. Evidence now suggests that this event was felt well beyond its place of origin in northwest Europe, although its manifestation varied regionally across the globe[5,6].

In inter-tropical Africa, the period of the Younger Dryas is marked by sharp regressions in the water level of lakes such as Bosumtwi (Ghana, 6° N), Chad (13° N) and Ziway-Shala (Ethiopia, 8° N), indicating negative hydrological balances (a net deficit of water) and an arid climate[7]. These same lakes also record later regressional (arid) events of apparently similar magnitude and duration to the one contemporaneous with the Younger Dryas, for example between 8.0 and 7.2 kyr ago. This 7.5-kyr event is broadly coincident with, and may have been caused by, the collapse of the Laurentide ice sheet over Hudson's Bay. Although ^{14}C chronologies indicate that the hydro-climatic events centred on 10.2 and 7.5 kyr ago were synchronous between different lake basins in Africa, they have seemed until recently to be geographically restricted to sites in the inter-tropical zone. But the investigation by Gasse *et al.* of the more northerly Sebkha Mellala lake basin (Algeria, 32° N), and similar studies on the Tigalmamine basin (Morocco, 33° N) (refs 8 and 9, and H. Lamb *et al.*, unpublished work), now reveal that these short-lived regressional events occurred beyond as well as within the tropics. The interruptions to the hydrological cycle at around 10.2 and 7.5 kyr ago are thus apparently of continental or even of global significance.

H. F. Lamb

Lake Tigalmamine, in the Middle Atlas mountains of Morocco. Pollen and diatom analyses of sediment cores[8,9] from this lake show evidence of abrupt short-term falls in lake level which occurred at times similar to those known from sites in tropical Africa.

One intriguing question, on which the jury is still out, is whether sub-Milankovitch climatic events have occurred throughout the recent geological past or whether they were restricted to times of major climatic transition, such as the end of the last glaciation. Abrupt lake-level oscillations are not equally 'visible' throughout the late Quaternary record: regressional events, for example, are stratigraphically much more evident at times of high rather than low lake levels. The record of the late Holocene (in any case a period of low lake levels in Africa) is complicated further by increasing human influence and the difficulty of disentangling natural from cultural factors. Nonetheless, data are beginning to emerge which suggest that tropical climatic 'crises' occurred not simply over recent millennia but even in recent centuries. One especially startling piece of evidence comes from cores taken in Lake Malawi, which reveal that the level of this great lake fell by about 100 m at some stage within the past thousand years, signifying a reduction in rainfall at that time to 50–70 per cent of modern values[10].

To understand the climatic mechanisms responsible for these hydro-climatic events, there is a need to establish as precisely as possible their spatial extent, age, duration and intensity. As Gasse *et al.* have shown, non-outlet lakes in regions such as Africa and the western United States look set to provide the best source of answers to such questions. It is clear that, unlike the Milankovitch changes brought about by variations in the solar radiation received by the Earth, these events cannot easily be predicted, thereby creating considerable uncertainty for projections of future climate. ☐

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NEUROBIOLOGY

One neuron, many units?

Larry Cohen and Jian-young Wu

Is the neuron the functional unit of the nervous system or can small portions of a neuron act as independent units? Neurobiologists have been speculating about this second possibility for more than 30 years[1,2], partly because microelectrode recordings from cerebellar Purkinje neurons reveal small spike signals that are almost certainly all-or-nothing events occurring in restricted areas of the dendritic tree[3,4]. Bill Ross, Nechama Lasser-Ross and Bob Werman[5] have now determined the spatial distribution of such events by using multi-site optical recordings with a calcium indicator dye. The findings are important because they show that one can now directly measure the independent activation of parts of a neuron.

One of the results obtained by Ross *et al.* is represented in Fig. 1, which shows a high-time-resolution recording from a Purkinje cell in a slice from guinea-pig cerebellum. Three dendritic regions can be differentiated: a region on the lower left where both of the small spike signals are detected as rapid increases in calcium; regions on the upper left and lower right where only the second of these signals is detected; and regions where neither signal is detected. In one especially complex cell, the authors were able to resolve six different compartments.

Because the dendrites of the cerebellar Purkinje cell are postsynaptic only, the effects of independent activation of the dendritic elements would be integrated at the spike-initiating zone, thereby determining the cell's output. This kind of organization is not, however, universal. Other neurons have both output and input synapses in the dendritic tree[6]. If a dendrite of such a neuron were independently activated, it would form a complete functional unit. It is possible, for example, that dendrites function independently in certain horizontal cells and starburst amacrine cells of the retina, and in nonspiking interneurons in the locust. Calculations suggest that the dendritic trees of these neurons can be divided into several electrically distant regions[7-10], and electron micrographs show that the independent regions both receive and make synaptic contacts. Furthermore, for both the horizontal cell[7] and locust interneuron[11] there is physiological evidence that is consistent with the existence of independent units, but this has not so far been directly demonstrated. Such a demonstration might best be achieved by measuring the membrane potential at many sites using a voltage-sensitive dye on the inside of the neuron: changes in membrane potential may be interpreted more easily than changes in calcium concentration. Unfortunately, however, the signals from voltage-sensitive dyes applied to the inside of neurons have so far been relatively small[12,13].

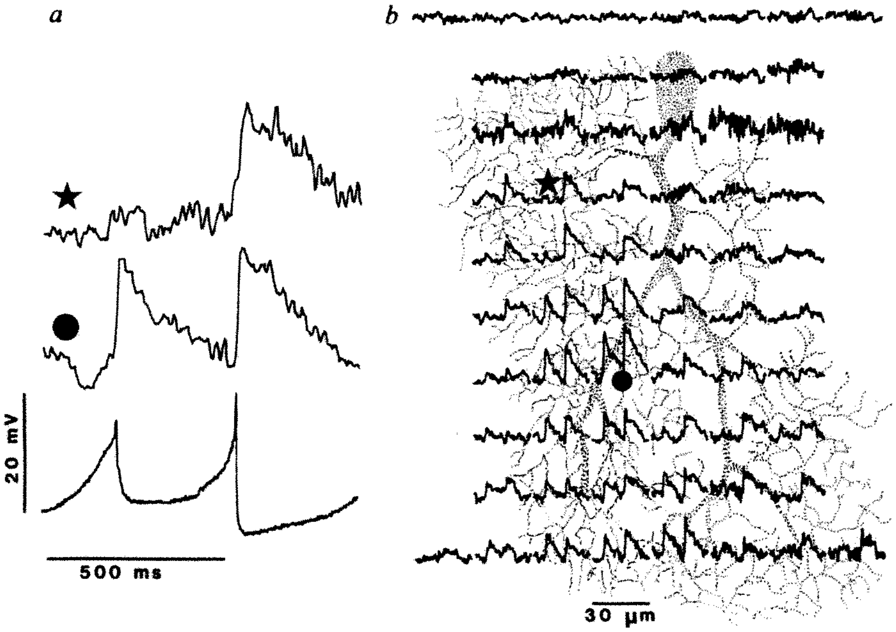

FIG. 1 Optical and microelectrode recordings from a Purkinje cell that has been microinjected with Arsenazo III in a 200-μm slice from guinea-pig cerebellum. *a*, The microelectrode recording from the soma (bottom trace) shows two small spontaneous spikes which differ in size. Optical recordings from detectors receiving light from two different areas of the dendritic tree are shown above the recording. In the top recording only the second spike event is detected, whereas in the middle trace both spike events are seen. *b*, Outputs of 64 photodiodes receiving light from 64 areas (each 30 × 30 μm square) of the slice overlaid on a drawing of the neuron. The cell body is at the top; the axon (not shown) leaves the cell body at the top of the figure. (Modified from ref. 5.)

It is difficult to predict how widespread independently acting dendritic units might be; there are neurons in which independent activity seems unlikely. For example, signals initiated anywhere in the electrically compact dendritic tree of an insect neuron can be detected equally in the cell body[14]. And in some crustacean neurons it has been impossible to detect compartmentalization of the calcium signal[15].

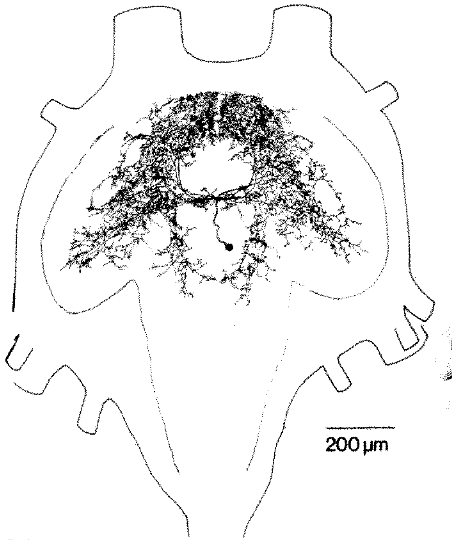

FIG. 2 Structure of a small dorsal unpaired median (DUM) spiking interneuron from a grasshopper ventral ganglion. The cell was stained with cobalt–silver and drawn from a whole mount. (Modified from ref. 19, courtesy of Karen Thompson and Melody Siegler.)

Although the prospect of directly measuring independently functioning regions of the dendritic tree is exciting for cellular neurobiologists, the existence of such regions would pose a problem for those trying to understand how behaviours are generated from neuronal interactions. Monitoring the activity of independent units in a dendritic tree is much more difficult than simply keeping track of the membrane potential of the cell body.

This would not be the first instance where an important discovery in cellular neurobiology increased the difficulty of understanding how behaviours are generated. Another was the demonstration that some neurons do not generate action potentials; these nonspiking neurons maintain their resting potentials in the middle of the range of potentials leading to transmitter release[2,6,16]. A problem in monitoring the activity of a nonspiking neuron is that the membrane potential measured at one location in the cell might not be an accurate representation of the potential at other locations in the cell.

Although nonspiking neurons are widespread in the animal kingdom[2], there are nervous systems in which such cells are relatively rare. Even though nonspiking neurons are found in gastropod molluscs[17,18], the best guess is that less than one per cent of the central neurons in *Aplysia* are nonspiking. Furthermore, nonspiking synaptic interactions are also rare in *Aplysia*.

A third instance of a double-edged success for cellular neurobiology is provided by the complex structure of neurons seen under the microscope. One beautifully ornate example is the interneuron from a grasshopper ventral ganglion illustrated in Fig. 2. The extent of the dendritic tree implies that the neuron may be in synaptic contact with a large number of other neurons in the ganglion. Obviously, a nervous system in which each neuron interacts with many neurons is more difficult to analyse than one in which synaptic interactions are restricted.

Widespread synaptic interactions may be the rule rather than the exception. For example, serial-section electron microscopy indicates that a mechanosensory cell in the leech makes about 2,500 anatomical synapses onto postsynaptic neurons[20] (K. Muller, personal communication); it would be interesting to know the distribution of the 2,500 synapses among the 390 neurons in the ganglion. In the nematode *Caenorhabditis elegans*, the ADE-L sensory neuron is monosynaptically connected to 9 per cent of all other neurons in the animal and disynaptically connected to an additional 52 per cent. Only slightly lower percentages are obtained starting with sensory neurons OLL-L and PHC-L (compiled from ref. 21). Finally, in *Aplysia*, 300–400 of the 1,000 or so neurons in the abdominal ganglion are activated by lightly touching the siphon skin[22,23]. It seems that information about sensory stimuli is propagated widely in these nervous systems. This raises the possibility that the organization of the response to the stimulus may also involve many neurons[24].

It will be exciting to see what roles these complex cellular properties have in the generation of behaviours. Using the techniques of Ross *et al.*, the existence of independent functional units in a neuron can now be directly studied. It will be a challenge to systems neurobiologists to develop methods for handling the potential complexity of widely interconnected neurons. But, surely, the question of how behaviours are generated from interactions of neuronal elements is the central concern of neurobiology. ☐

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EVOLUTIONARY EPIDEMIOLOGY

Parasite clones in the wild

Anne E. Keymer, Robert M. May and Paul H. Harvey

SEVERAL species of parasitic protozoa will reproduce sexually in the laboratory[1-3]. That sexuality is an advantage in such cases is indicated by an excess of recombinants being produced from mixed-genotype infections[4]. It does not follow, however, that sexuality is important in the wild just because it can occur and have advantages in the laboratory. Indeed, preliminary field observations of several species (including the protozoa that cause leishmaniasis and Chagas disease) have indicated clonal population structures that are stable for many generations and over wide geographical areas[5,6]. Until recently it could be assumed that the laboratory and field studies had looked at different species or different strains of a species, and that sexual reproduction differed in frequency between the two groups. But a new report by Tibayrenc and colleagues in *Proceedings of the National Academy of Sciences*[7] casts doubt on this interpretation: species which may be sexual in the laboratory have a clonal population structure in the wild. The finding may have important implications for biological control programmes.

Using data from species belonging to seven genera of parasites, Tibayrenc *et al.* propose that clonal population structures are the norm in natural populations. In each genus (*Entamoeba, Giardia, Leishmania, Naegleria, Plasmodium, Trichomonas, Trypanosoma*) there are fewer genotypes than would be expected if regular outbreeding occurred. In their analyses of biochemical variability, the authors used seven criteria when testing for clonality. Three are based on tests of the significance of observed levels of segregation and are applicable only to diploid organisms: fixed heterozygosity, the absence of segregation genotypes, and deviation from the expectations of a simple Hardy–Weinberg equilibrium model. The other four criteria are tests of the significance of observed levels of recombination and are thus independent of ploidy: widespread existence of identical genotypes, absence of recombinant genotypes, linkage disequilibrium, and correlations between independent sets of genetic markers. The results imply that naturally occurring populations derive from the clonal reproduction of a few highly successful genotypes, as highlighted by the predominance of particular clones in different localities, sometimes in different host species, and at intervals separated by many years.

A few examples illustrate the new findings. The *Leishmania* zymodeme (a genotype determined by electrophoretically distinguished enzyme patterns) MON 1 is predominant in both the Old World and in Latin America. A clone of *Plasmodium falciparum* (Gpi1/Ada1/Ldh1/Pep1) has been isolated from ten out of 17 samples in five countries spanning Africa and Indochina. *Entamoeba histolytica* zymodemes I, II and III have been sampled repeatedly in Canada and South Africa. Six identical strains of *Trichomonas vaginalis* have been identified: five that were isolated recently, and one in 1939. And a single zymodeme of *Giardia* has been found in five human hosts and two cats (one in Western Australia and the other in Oregon).

The results imply that clonality should be adopted as the working hypothesis by those involved in research on diseases caused by the parasitic protozoa. Genetic

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recombination is not ruled out — the data simply show that it does not significantly affect the prevailing patterns of clonal population structure. What, then, of the sexuality so clearly demonstrated in the laboratory? Are recombinants selected against in the wild?

More ambitiously, can these systems provide us with precise, quantitative insights into evolutionary problems such as the generation of stable or shifting polymorphisms by frequency-dependent selection, or the origins and maintenance of sexual reproduction[8,9]? To answer these questions, we need to know more about the epidemiology of individual clones, the frequency of multiple-clone infection, and the mechanisms and consequences of clonal competition. Increased integration of work in the field and in the laboratory will help, as will further cooperation between geneticists and epidemiologists. ☐

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SUPERCONDUCTIVITY

Bringing about some order

Robert J. Cava

It seems fitting that $YBa_2Cu_3O_7$, the first material found to be superconducting above liquid-nitrogen temperature, exhibits one of the most interesting and complex interplays between chemistry, crystal structure and physical properties of any ceramic material studied so far. At the heart of the matter is a subtle charge balance between the one-dimensional copper–oxygen chains, which have variable oxygen content, and the two-dimensional copper–oxygen pyramidal planes, where superconductivity originates (see figure). The latest surprise is described in an elegant paper by Jorgensen *et al.*[1], who find a correlation between the appearance of superconductivity in an originally nonsuperconducting oxygen-deficient sample, after annealing at room temperature, and time-dependent changes in its crystal structure. As well as demonstrating that the oxygen atoms are mobile at ambient temperature, the results show more importantly that the superconducting properties are remarkably dependent on local, short-range structure. This situation is likely to be unique to the high-transition-temperature copper oxides, in which the coherence lengths of the superconducting charge carriers are of the order of the size of the crystallographic unit cell.

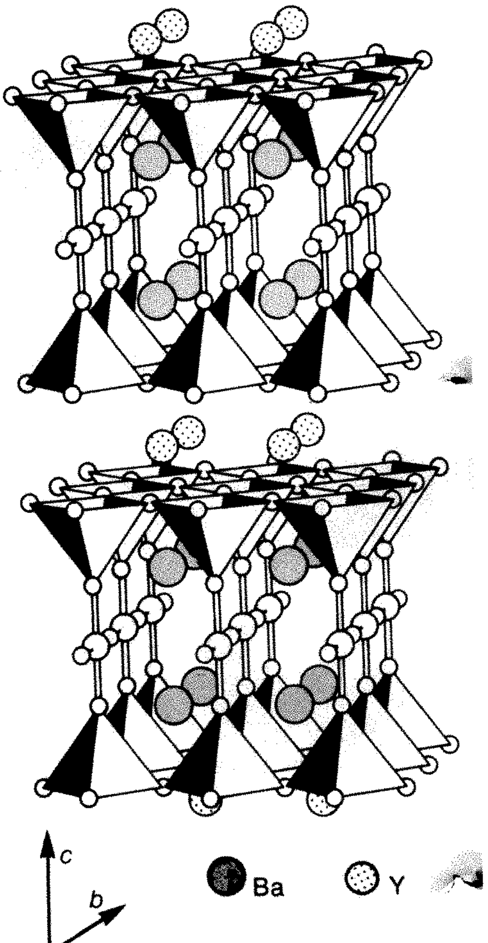
In oxygen-deficient $YBa_2Cu_3O_{7-x}$, oxygen is removed from the Cu–O chains: a 90-K superconductor is obtained for $0 \leq x \leq 0.2$, a 60-K superconductor for $0.3 \leq x \leq 0.55$, and an antiferromagnetic insulator for $0.55 \leq x \leq 1.0$. These changes can be shown to correspond to a series of charge transfers from the chains to the pyramidal planes[2]. In $YBa_2Cu_3O_7$, the Cu–O chains are aligned along one crystallographic axis (the orthorhombic *b* axis), while an equivalent direction in which they could lie — the *a* axis, perpendicular to the *b* axis — remains unoccupied (thus the *a* axis is shorter, as the chain oxygens are missing; see figure). As oxygen is removed, the chains become fragmented; eventually the fragments become short enough to be able to align along either the *a* or the *b* axes. These axes then become equivalent, making the overall symmetry tetragonal even though on the scale of the length of the chain fragments the symmetry is still orthorhombic. The orthorhombic-to-tetragonal phase transition is a function of both the total oxygen content and the temperature, because the orientational disordering of the chain fragments increases the entropy of the system.

The paper by Jorgensen *et al.* follows up their initial discovery[3] of time-dependent superconducting phenomena in $YBa_2Cu_3O_{7-x}$. The present results were obtained from polycrystalline samples prepared at the composition $YBa_2Cu_3O_{6.41}$ by annealing $YBa_2Cu_3O_7$ for a long period at 520 °C at reduced oxygen pressure and then quenching into liquid nitrogen. At the preparation temperature and oxygen content, the compound is tetragonal with disordered chain fragments. The quenching is performed to maintain the sample as much as possible in the high-temperature disordered state, which should not be stable with respect to lower-entropy states at room temperature. But the orthorhombic symmetry observed for the samples immediately after quenching indicates that even at a rapid quench rate the oxygen atoms have time to partially rearrange from the disordered structure.

One of the clever aspects of these experiments is that Jorgensen *et al.* pick an oxygen content right at the superconducting/nonsuperconducting boundary. The sample is initially nonsuperconducting, but soon develops a superconducting transition with a transition temperature that saturates at 20 K after about 72 hours. This temperature is significantly lower than the 55–60 K observed for 'equilibrium' samples at a composition near $YBaCa_2Cu_3O_{6.50-6.45}$ (which corresponds to the minimum oxygen content generally required for superconductivity).

In a demonstration of one of the great assets of the neutron time-of-light powder-diffraction technique, the authors have made full crystal-structure determinations at 27 stages during the first 26 hours of the ambient-temperature annealing. The structural changes involve the shortening of all the crystallographic axes, with the greatest shrinkage along the *a* axis, and also a shortening of the bond between the pyramidal copper and its apical oxygen. Surprisingly there is no change (within the precision of the measurements) in the amount of oxygen in the chain sites on the *a* axis relative to the those on the *b* axis, implying that there is no large-scale lengthening or increase in the number of chain fragments aligned along *b* at the expense of those along *a*. In addition, there is no change in the total oxygen content.

The authors estimate the length scale of the diffusion process that gives rise to these structural and physical changes to be 4 Å — approximately the size of a unit cell. The appearance of superconductivity, therefore, is due to the short-range



The structure of $YBa_2Cu_3O_7$, showing the one-dimensional Cu–O chains and the two-dimensional Cu–O pyramidal planes. Crystallographic *a*, *b* and *c* axes are also shown.

rearrangement of oxygen atoms in the chains, giving rise to charge transfer to the planes as is evidenced by the decrease in the distance between the plane copper atoms and the apical oxygen. Because of the very short coherence lengths of the superconducting charge carriers, superconductivity is observed even when only microscopic regions of the crystallites have the appropriate oxygen configuration. The relatively low transition temperature may be due to the proximity of superconducting and nonsuperconducting regions. It is not easy to understand, without more data, exactly how a microscopic rearrangement of oxygen atoms can produce no change in the overall ratio of the *a*- to *b*-orientated chain fragments while disproportionately decreasing the *a*-axis length. Perhaps, as Jorgensen *et al.* suggest, it involves the formation of alternating, parallel full-chain/empty-chain fragments on a local scale, as are found on a longer scale for the 60-K superconductor $YBa_2Cu_3O_{6.5}$. But whatever the case, whereas some might argue that there can no longer be anything new or exciting to learn about $YBa_2Cu_3O_7$, Jorgensen *et al.* have clearly shown otherwise. ☐

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OCEAN DRILLING PROGRAM

Reading the ocean's diary

Leg 130 shipboard scientific party

How does sedimentation respond to changes in global climate and ocean dynamics? And what is the origin of the world's largest oceanic plateau? It was to address these questions that Leg 130 of the Ocean Drilling Program took the RV *JOIDES Resolution* to the Ontong Java Plateau in the western equatorial Pacific. This plateau is ideally suited to such studies, being a flat, shallow region of sea floor — the result of an unusually thick (40-km) oceanic crust and a thick (1,400-m) sediment cover. Being free of interference from coastal ocean processes, the region has the potential to provide a complete, undisturbed record of open-ocean pelagic sedimentation since the middle Cretaceous (about 100 million years ago). The cores recovered by Leg 130 succeeded not only in illuminating this history, but also in supplying clues to the origin of the plateau itself.

The Ontong Java Plateau (Fig. 1) has drawn the interest of marine geologists since the beginnings of deep-sea drilling[1–3], and has provided standard sedimentary sections for the evaluation of Pleistocene palaeoceanography[4,5]. Two boreholes drilled during Leg 130 penetrated through the sediment cover into the basalt basement below (one to a depth of 26 m and the other to 149 m into the basalt), opening the way for petrological studies of the origin of the plateau and for palaeomagnetic studies of its movement since the middle Cretaceous. The basements of oceanic plateaus in the Pacific have been penetrated only four times by previous drilling projects, and never before to such depth. The Leg 130 results show that the Ontong Java Plateau is oceanic in origin, having been formed at depths of 2,500–3,000 m during the early Cretaceous from flood basalts and flows of basaltic pillow lavas. One of the significant aspects of oceanic plateaus (of which the Ontong Java is the largest, being one quarter of the size of Australia) concerns their possible role in the growth of continents[6]. Palaeomagnetic data will provide an important means of tracing their movements throughout past epochs, as all of the drill sites on the Ontong Java Plateau reached their present equatorial locations only relatively recently (during the late Neogene — the past 5 million years). Massive outpourings of basalt during the early Cretaceous, of the sort that now seem to have formed the Ontong Java, have been postulated before[7]. They would have caused changes in global sea level and — if occurring over a sufficiently short time interval — also in oceanic circulation and chemistry.

But the principal palaeoceanographic questions addressed during this leg concerned the response of the ocean — specifically the biogenic sedimentation therein — to changes in the variation of mean temperatures across the planet. It is known that during the Neogene, the temperature gradient between the equator and the poles increased as a result of expanding snow and ice fields in polar regions. This trend resulted from thermal isolation of the Arctic Ocean and the Antarctic continent (through continental drift) and from overall sea-level regression. Regression is the consequence of mountain building, which thickens continental crust and deepens the ocean basins; it exposes land surfaces, which reflect more sunlight than the sea, and thus increases the planetary albedo.

The way that the global oceans responded to this increasing temperature gradient would have been mediated by the major changes in geography that were taking place at that time — the opening of the Drake Passage between South America and Antarctica, the intermittent build-up of the Antarctic ice cap, the closing of the Tethys seaway (which once connected the Atlantic and the Pacific from the Mediterranean region to the Himalayas), the rise of the Himalayan mountains and the separation of the Pacific from the Atlantic by the Isthmus of Panama. The combination of this evolving geographic setting and the increasing latitudinal temperature gradient would result in an episodic reorganization of established patterns of heat flow and ocean productivity, and thus in episodic changes in plankton distributions and in sedimentation of biogenic matter.

The Leg 130 sediment cores gave insight into these complex processes. The sedimentation-rate profiles for each site (Fig. 2*a*) show that the overall sedimentation rate for the past 25 million years is dominated by a low in the late early to early middle Miocene (20 to 15 Myr ago) and a prominent peak in the late Miocene to the earliest Pliocene. The crossing of the equator by some sites (indicated by squares in Fig. 2*a*) had no or little influence on these trends. At sites 803 and 804, the periods of low sedimentation rate are characterized by hiatuses (denoting levels where sediment is missing), suggesting sporadic sediment removal by erosion or mass movement[8]; but sites 805–807 seem to be biostratigraphically complete. The onset of the low-rate period coincides with a marked change in the carbon

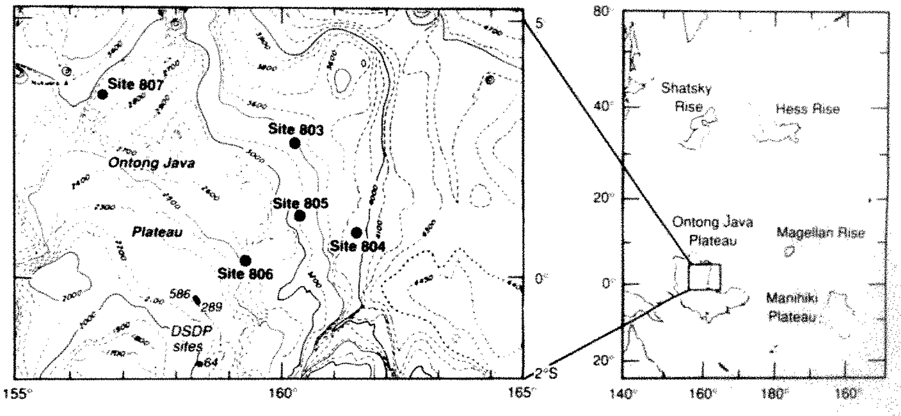

FIG. 1 Location map of Leg 130 sites. Bathymetry is in metres.

isotope composition of the ocean[9], which is the result of an increased difference in productivity between the deep sea and coastal waters. Increased coastal productivity captures a greater share of nutrients for the margins, with the result that sedimentation in the deep sea is decreased.

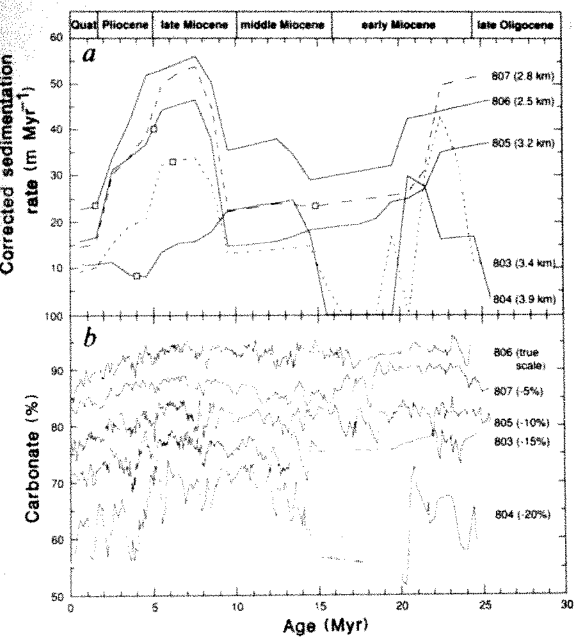

FIG. 2 *a*, Sedimentation rates (corrected for compaction) as a function of age for Leg 130 sites 803–807. Water depth for each site is indicated on the far right-hand side. Squares indicate the times when the sites crossed the equator. *b*, Carbonate percentages as a function of age for Leg 130 sites 803–807. Note that the absolute carbonate values for the sites have been adjusted by 5–20 per cent (see right-hand side of figure) to facilitate comparison. Acoustic reflectors in the seismic profiles often coincide with sharp changes in carbonate content.

The period of high sedimentation rate, centred around 7 Myr, in fact displays some of the highest rates (about 50 m Myr^{-1}) ever recorded in open-ocean pelagic sediments. The timing of this period suggests that these high rates may be associated with the closing of the Tethys and with important phases of mountain-building, leading to an increased supply to the ocean of continent-derived materials. At this time, a pronounced gradient of productivity developed from east to west in the equatorial Pacific[10,11]. Eventually the piling up of warm water in the western Pacific, owing to increased trade winds, led to a reduction of productivity. The high sedimentation rates fell off during the Pliocene and especially during the Quaternary, accompanied by decreasing preservation of diatoms and radiolarians in the sediments. This drop also may be the result of removal of nutrients and silica along the coastal oceans by increased coastal upwelling. This finding is puzzling because we must assume that strong trade winds continued to drive upwelling in equatorial regions, and yet the sedimentation rate dropped at the sites drilled.

Since the middle Miocene, sedimentation rates at all the sites have been similar. The amounts of carbonate deposited (Fig. 2*b*) are also highly correlated between sites for the past 12 million years, but poorly correlated before this. These observations suggest that a strong vertical gradient of carbonate saturation, which controls the increase of dissolution of carbonate with depth, became established at this time as a result of increased cold deep water formation. But it is puzzling that the carbonate values of deep sites are so similar to those of shallow sites. Carbonate removal by dissolution cannot by itself explain simultaneously the (distinct) differences in sedimentation-rate patterns between the sites, and the (slight) differences in carbonate content. More will be learned on this point by improving the resolution of sedimentation rates through analyses of Milankovitch frequencies in the record, of physical properties and of stable isotopes.

An important objective of Leg 130 was to account for the strong acoustic reflectors that give a 'layer-cake' appearance to seismic profiles of the sediments. Many of these reflectors correspond to the sharp changes in carbonate content seen in Fig. 2*b*, and there is some correlation between sites. On the whole, the results support the proposition that at least some of the major reflectors are caused by palaeoceanographic events (such as dissolution pulses) that affect carbonate deposition[12,13]. ☐

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DAEDALUS

Animal warmth

Animal fur and feathers are wonderfully self-adjusting. An animal or bird can fluff up its coat in cold weather for extra insulation, or smooth it down in the warm to dissipate heat. Daedalus is now imbuing human clothing with the same thermostatic cunning.

He is exploiting the 'bimetallic strip' principle: a laminate of two materials with different coefficients of expansion curls with change of temperature. Two-component synthetic fibres are already well known, so it should be easy to bond two polymers of contrasting expansion coefficients, like nylon and polyester, into a highly thermosensitive fibre. Inserted as flock or tufting in a conventionally woven fabric, such fibres will form a velvety 'Cat-fur'. Its pile will fluff up in the cold, but curl over and lay flat in the heat, neatly thermostatting the wearer.

DREADCO's Cat-fur will come with a range of transition temperatures. For coats and other outerwear, it should fluff up at moderate outdoor-air temperatures, so as to respond to changes in the weather. A higher-temperature grade will be used in underwear. Responsive to typical skin temperatures, it will maintain intimate comfort in the face of wide swings of metabolic rate, from the hot sweat of exertion or anxiety to the chill of desperate foreboding.

The ultimate in thermostatted clothing will be a complete one-piece 'Cat-suit'. Each element of its surface will incorporate the right mix of Cat-fur fibres to maintain the skin beneath at its optimum temperature. It will be equally comfortable indoors or outdoors, in winter or summer, in violent exercise or total relaxation. The wearer will need no other garment!

An extreme test of Cat-fur will be at night, in its role as pyjamas or bed-clothes. A sleeper produces so little heat that he needs the maximum of thermal insulation; even so, modern beds (especially duvets) probably overdo it. A time-lapse film of a sleeper looks like a fight with an invisible boa constrictor, but is really a constant search for thermal equilibrium. When overheated, the sleeper must move to a cooler part of the bed, which soon heats up in its turn; when too chilly, he must curl up to conserve heat. This ceaseless unconscious activity is far from restful.

In blissful contrast, a sleeper insulated by DREADCO's Cat-fur will be in perfect thermal equilibrium all through the night. He will never have to move, and will truly 'sleep like a log'. A whole new era of total nocturnal comfort and perfect sleep will be ushered in! The dreary syndrome of daytime exhaustion, with its gummed eyelids and drained enthusiasm, will be abolished; instinctive zest for life, long-forgotten by the legions of Insomniacs Anonymous, will be restored.

David Jones

Link between scrapie and BSE?

SIR—Peter Aldhous recently reported[1] the view that transplacental passage of scrapie infectivity occurs in scrapie-infected ewes. But there are contradictory reports concerning this issue, reflecting our ignorance about the origins, spread and pathogenesis of natural scrapie. The elusive aetiology of scrapie underscores the difficulties encountered in designing and interpreting studies. As we describe here, some view natural scrapie as a viral-like illness whereas others describe it as a genetic disorder that happens to be experimentally transmissible.

Over the past 5 years, molecular biological and genetic data have profoundly altered our views of experimental scrapie in rodents and the trinity of human prion diseases — kuru, Creutzfeldt–Jakob disease (CJD) and Gerstmann–Sträussler syndrome (GSS). Indeed, the application of modern techniques to this enigmatic area of biology has begun to provide explanations about how a disease can be both genetic and experimentally or iatrogenically infectious[2,3]. The main, and possibly only, component of the transmissible particle (or prion) in scrapie is an abnormal isoform of the host-encoded prion protein (PrP)[4], which is generated by an as yet undefined post-translational process. That PrP has a central role in scrapie transmission and pathogenesis is independently supported by studies on the purification of scrapie prions, on the molecular genetics of scrapie incubation time genes and by analyses of infected neuroblastoma cells[3].

Maternal (and lateral) contagious transmission of natural scrapie was first suggested by crosses of scrapied and scrapie-free Suffolk sheep. Progeny of affected ewes rather than rams were about seven times more likely to develop the disease[5]. In a subsequent study of Suffolks crossed with Scottish Blackface sheep a similar but reduced tendency was again apparent; ratios of approximately 1.9 and 1.3 to 1 were obtained in experiments involving 54 and 66 offspring, respectively. A 'background' scrapie incidence of up to 50 per cent in the progeny of unaffected parents, interpreted in terms of lateral contagious transmission, was apparent in this later study[6]. A tendency favouring maternal transmission in Parry's data[7] on twins born of scrapie-infected ewes was noted by Dickinson *et al.*[5], although the numbers quoted for the progeny of unaffected ewes crossed with affected rams do not seem to correspond to the original data. But most of Parry's data on Suffolk sheep revealed no overt tendency for ewes rather than rams to transmit the disease[7]. Parry also found no evidence for contagious transmission — scrapie-free animals produced no affected offspring, even when crossed with scrapied animals.

The hypothesis of maternal transmission can be directly tested by bioassay for infectivity in scrapied ewes. Hadlow and coworkers failed to detect infectious titre in the uterus, ovary or mammary gland of clinically affected Suffolk ewes[8]. Two papers[9,10] have been widely cited as indicating infectivity in the placentae of Swaledale ewes with scrapie. Unfortunately, negative controls for these experiments were not described and the incubation times in the inoculated recipients were scattered; an alternative explanation is that the observed instances of scrapie represent cross-contaminated inocula (but see ref. 15).

A few cases of transmission from experimentally inoculated ewes to offspring been observed[11,12], but in an embryo transfer study by W. Foote *et al.* (personal communication) none of the offspring developed the disease within an observation period greater than or equal to 5 years ($n = 86$). Direct inoculation of control animals in these experiments produced a scrapie incidence of more than 51 per cent. Similarly, negative results were obtained for maternal transmission in experimental scrapie of goats[13]. Early reports suggesting maternal transmission of scrapie in mice have subsequently been strongly challenged. A maternal effect is not apparent in the transmission of either familial or experimental CJD, kuru or GSS.

In summary, the question of maternal transmission represents part of the larger issue of whether natural scrapie in sheep is a contagious disease under normal conditions of husbandry. As bovine spongiform encephalopathy (BSE) is inferred to have arisen by oral-dosing with contaminated feedstuff[14], and given that some scrapie researchers considered that natural and experimental scrapie were not necessarily identical diseases[7,15], the data on natural scrapie may be irrelevant to the anticipated behaviour of BSE. But as the dataset on experimental scrapie contains evidence both for and against 'maternal' transmission, and as there is a potential human health hazard concerning BSE, the resolution of this matter must come from future experimentation. Because our studies with transgenic mice[16] show that the species barrier for passage of scrapie prions between rodent species is likely to reside in the amino-acid sequence of PrP, similar experiments seem relevant in examining the potential for transmission of prions from beef and sheep products to humans.

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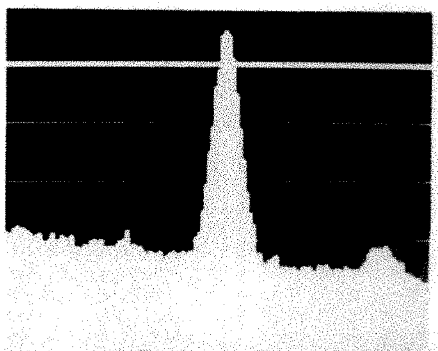
Frustrated crystallographers

SIR—Dover's News and Views article[1], indicating that "many frustrated crystallographers are left wondering why their crystals will not grow large enough or well-ordered enough, or grew yesterday but will not grow today", brought back memories of my own experiences with this problem. When I first joined Professor Tom Blundell's group, good-sized crystals of the newly discovered avian pancreatic polypeptide hormone (aPP) had just been grown by Dr Steve Wood. But the small pot of crystals was soon depleted by low-resolution data collection and by screening more than 30 heavy-atom soaks by precession photography.

Subsequent laborious preparation of new materials and numerous crystallization trials would no longer result in crystals. Suspecting the presence of an unknown co-factor, I analysed the crystals using scanning electron microscopy. The microscope was fitted with an EDAX system which could potentially be used to identify trace elements by measuring their



EDAX spectrum from an aPP crystal indicating the presence of zinc.

characteristic X-ray emission energies.

I placed one of the remaining small crystals of aPP on a sample stub to air dry and to my surprise it did not disintegrate. After coating it with carbon, I examined the sample under vacuum in the microscope and it remained crystalline in appearance. I recorded EDAX spectra over a 400-s time period and observed a peak at 8.63-KeV characteristic of the K_α line for zinc. The addition of zinc acetate to the crystallization experiments resulted in much larger crystals, indicating that the original scavenged zinc must have been limiting[2]. The resulting high-resolution X-ray analysis indicated the key role this divalent ion played in forming the crystal lattice[2,3].

I have also applied this technique to porcine 2-zinc insulin crystals and to freezed-dried iron containing transferrin. The ability to detect and identify traces of heavy elements acting as essential cofactors in protein crystals may provide one escape route for the baffled crystallographer.

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Expanding family

Sir—We have recently described[1] a family of six fungal genes involved in mitosis and the regulation of RNA synthesis whose protein products have in common a repetitive sequence motif of 34 amino acids (tetratricopeptide repeat or TPR). Zhang *et al.* (manuscript in preparation) subsequently found 16 tandem TPR sequences in the *crooked neck* (*crn*) gene involved in neurogenesis in the fruitfly *Drosophila*. We have now identified two additional family members in the yeast *Saccharomyces cerevisiae*.

The product of one gene, first cloned in 1983 (refs 2, 3), is a protein of relative molecular mass (M_r) 70,000 and was used for studying the targeting of mitochondrial proteins encoded in the nucleus. The 41 amino acids at the amino terminus anchor the protein to the outer mitochondrial membrane and the rest lies in the cytoplasm. It is this portion that contains seven tandem TPR sequences; this arrangement of TPR repeats is remarkably similar to that of the cell-division cycle (CDC) subset of the TPR family[1]. It is known that a truncated version of the gene, whose product is shortened at the carboxy terminus by 203 residues, including almost all of the tandem TPR domain, is anchored normally but produces cells with the same mutant phenotype as that of cells that lack the protein. Could it be that the repeated sequence domain mediates the interaction of mitochondria with the cytoskeleton much as *nuc2*$^+$ is thought to interact with the nuclear 'scaffold'[4]?

The product of the second gene, *STI1*, is a protein of M_r 66,000 induced by stress but not similar to the HSP70 family of heat-shock proteins[5]. Again there are internal TPR sequences, but of variable length, in an arrangement resembling that of the product of SKI3 gene involved in RNA regulation.

The inferred amphipathic helical structure of the TPR unit[1,4] suggests several possible functions, including protein–lipid interaction and the mediation of protein dimerization. Our recent (unpublished) finding of intragenic complementation between temperature-sensitive alleles of *CDC23* by mutations in different TPRs suggests that protein-protein interactions are necessary for *CDC23* function.

In our original TPR dataset[1], we identified two more highly conserved subdomains, A and B, corresponding to the 'hole' and 'knob' in the repeat–repeat interaction model of *nuc2*$^+$ function[4]. In the new examples, the B submotif is maintained but there are different patterns of sequence conservation in A. We believe that TPR genes have evolved different functions based both on divergence of TPR sequences and also on the acquisition of separate, unrelated functional domains such as the targeting/anchoring sequence in the M_r 70,000 outer mitochondrial membrane protein and the *zeste*-like domain in *SSN6* (ref. 1). Consistent with this idea, we have now found that single amino-acid substitutions in *CDC23*, which cause a temperature-sensitive phenotype, cluster into two separate domains: the carboxy-terminal TPR sequence block and an amino-terminal domain of unique, unrelated sequence.

a

```
                                                                    residues:
A L A L K D K G N Q F F R N K K Y D D A I K Y Y N W A L E L K E D - ( 99-131)
P V F Y S N L S A C Y V S V G D L K K V V E M S T K A L E L K P D Y (132-165)
V N S Y I Y M A L I M A D R N D S T E Y Y N Y F D K A L K L D S N N (363-396)
S S V Y Y H R G Q M N F I L Q N Y D Q A G K D F D K A K E L D P E N (397-430)
P E V P N F F A E I L T D K N D F D K A L K Q Y D L A I E L E N K L (465-498)
L V G K A T L L T R N P T V E N F I E A T N L L E K A S K L D P R S (508-541)
E Q A K I G L A Q M K L Q Q E D I D E A I T L F E E S A D L A R T M (542-575)
                              *   *   *           * *   * *
```

b

```
                                                                    residues:
A D E Y K Q Q G N A A F T A K D Y D K A I E L F T K A I E V S E T P (  5- 38)
S K G Y N R L G A A H L G L G D L D E A E S N Y K K A L E L D A S N ( 74-107)
A D K E K A E G N K F Y K A R Q F D E A I E H Y N K A W E L H K D I (262-295)
S K S F A R I G N A Y H K L G D L K K T I E Y Y Q K S L T E H R T A (336-369)
                D I L T K L R N A E K E L K K A E A E A Y V N P E K (370-395)
A R G Y S N R A A A L A K L M S F P E A I A D C N K A I E K D P N F (430-463)
              V R A Y I R K A T A Q I A V K E Y A S A L E T L D A A (464-490)
                          L Q E H M K N P E V F K K - I Q T L I A A (563-583)
              *   *     * *           *       *   * *   *
```

c

```
              A                                   B
      _______________                 _________________________
A E A W F G L G H I Y E K L G D L E K A L D A F Q K A L E L D P N N   TPR
V K L W I K Y A R F E E L L K E I D R A R E I Y E R A L E F L P R D   crn
P V A Y I D L A Q I N F D V E D F D E A I K L F D K A L E L D P D N   OMMP
A K G Y A R E G A A L A K L G D A D E A I E D Y K K A L E L D P A A   STI1
                          *   *   *   *           * * * * * * *
```

Internally repetitive sequences and their statistical significance were identified and characterized by standard methods as described in ref. 1. The M_r 70,000 protein and *STI1* have intra-sequence comparison scores of 7.09 and 12.37 s.d. units, respectively; inter-sequence comparisons with other members of the TPR family yielded scores ranging from 3.44 to 12.43 s.d. units. Only the repeats of highest significance are shown. Starred columns, the most highly-conserved positions in the aligned sequences. The original TPR motif was based on 47 repeats from five different proteins and showed two more highly conserved subdomains labelled A and B and represented by the sequences W..LG..Y and A...(Y/F)..A...P, respectively (ref. 1). The CDC subset of TPR proteins includes *CDC23, CDC16* (*S. cerevisiae*), *nuc2*$^+$ (*S. pombe*) and *bimA* (*A. nidulans*); the RNA regulation subset includes *SKI3* and *SSN6* (*S. cerevisiae*). To facilitate comparisons with new candidate TPR genes, a file containing all known TPR protein sequences, as well as suggestions for quantitative comparisons, is available from M.S.B.

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Proportional time

Sir—If there is unease with the idea of a defined moment for the Big Bang, with no temporal predecessor, why not adopt E. A. Milne's logarithmic proposal, where $\tau = t \log(t/t_0) + t_0$, with τ as dynamical time and t as conventional time (*Proc. R. Soc.* **A158**, 324–348; 1937)? As Milne remarks, the epoch of creation t=0 on the kinematic scale is measured by $\tau = -\infty$ on the dynamical scale.

Indeed, more generally there is a case for thinking of time in proportionate rather than in arithmatically progressive terms. In sensory physiology, strength of sensation is usually proportional to log(stimulus); in pharmacology, drug effect is usually proportioned to log(dose); and on a wider canvas, wage negotiations, inflation, growth and what-not, are almost always expressed in proportionate terms. Why should the philosophy of time restrict itself to an unnatural arithmetical scale?

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Historical light on photography

SIR— During the historical session of the French Academy of Sciences on 19 August 1839, F. Arago disclosed the now well-known daguerrotype process invented by Daguerre[1]. But a few years earlier, in 1816, Joseph Nicéphore Niépce obtained the first negative picture on a paper coated with silver salts and placed in a crude camera obscura[2]. The picture was sent in a letter to his brother, but it was not fixed, and he also failed to produce a positive from it.

Niépce investigated numerous light-sensitive compounds which he classified into three types with respect to the effect of light: "colouring properties, discolouring properties and solidifying properties". He eventually succeeded in irreversibly fixing on a support the reflection of light from a landscape by using asphalt spread on glass, stone or metal. In 1829 he described his process which was published 10 years later[1]. Niépce had discovered that bitumen hardens on exposure to light, thus making it possible to capture the imprint of light and shadow of an image by removal of the unexposed areas with an appropriate solvent.

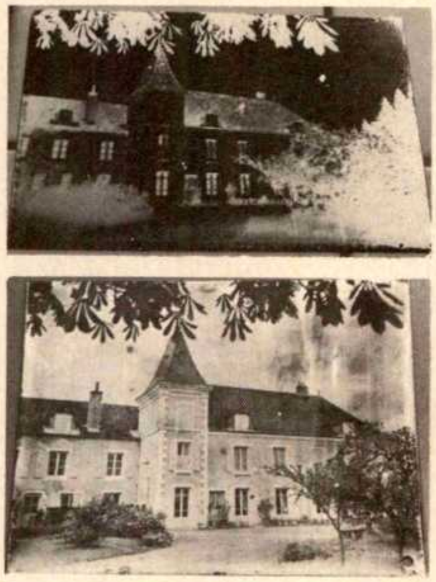
But the process, termed heliography by Niépce, remained obscure. Only a unique picture called *Point de vue du Gras*, obtained by Niépce in 1826, is known and his work cannot easily be evaluated from this single heliograph.

We have attempted to reproduce Niépce's technique and in June 1989 were able to produce the first heliograph ever made since Niépce's time[3]. We followed all his written methods, and experimentally determined the exposure time, which he never mentioned. The heliographic process is, in practice, rather different from the interpretation given by historians and provides a clearer understanding of some of the explanations given by Niépce in his letters.

The photosensitive compound used by Niépce was bitumen of Judea, or asphalt[4]. In Niépce's process, bitumen is dissolved in oil of lavender and spread as a thin layer on a well polished silver-coated plate as a varnish. This constitutes a photosensitive film which, under the action of light, partially loses its properties of solubility due to a crosslinking process[5]. This variation of solubility with dose allows one to dissolve the areas where the varnish remains soluble — those which have not been illuminated — while it becomes insoluble in the exposed areas.

The bitumen we used is a commercial product (Prolabo) in the form of a brown powder. Our preparation of the well-polished silver plate, covered with the bitumen varnish to obtain a thin golden brown film follows exactly the operations described by Niépce[1]. The optical absorption spectrum of a thin film of bitumen prepared from a bitumen solution (3 g in 10 ml of lavender oil) shows an optical density at wavelength's under 500 nm.

In the literature of the history of photography, the formation of an image by the Niépce process is explained as the simultaneous hardening and whitening of the bitumen under the action of light and thus would be a direct positive process[2,6-8]. Our first experiments were performed by irradiating homogeneously the light-sensitive plate either with sunlight or with a halogen lamp whose light intensity was controlled; the bitumen hardened under these conditions. But no change in the golden brown colour was detected even after a long irradiation with visible or ultraviolet light. Niépce said that no colour variation is observable after exposure to light. There is no whitening of the bitumen with light, contrary to what has frequently been claimed[1,2,6-9].



Top: negative image obtained with bitumen of Judea by using contact printing (see text). Light areas correspond to bright silver, dark areas consist of crosslinked brown bitumen. Bottom: direct reaction of iodine vapours on the negative image leads to the formation of silver iodine only where the bright silver of the negative image was apparent. Under the action of light these bright areas turn black. After complete removal of the bitumen still present, the positive image is obtained. (*Le Gras*: Niépce's house at St Loup de Varennes near Chalon-sur-Saône, France).

Niépce's method of obtaining a positive image is the following: the unprotected bright silver is blackened by submitting the negative plate to iodine vapours. This leads to the formation of silver iodine at the surface of the plate which is then transformed, under daylight, into finely divided black silver. The last operation consists of stripping the hardened bitumen off the plate with alcohol. In its final form, no trace of bitumen is left on a heliograph. The bitumen plays the role of an intermediate compound, like a negative image laid on silver. Iodine vapours penetrate the different areas of bitumen, proportional to their density, and restore all the variations of greys from black to white. The shadows are made of black colloidal silver, while the lights are polished silver. The image we obtain has a high resolution (80 lines per mm) and good reproduction of shades of grey.

Our second important result concerns the exposure time. Many hypothesis have been suggested[2] as Niépce never clearly mentioned this parameter. Three different methods have been used to obtain heliographs: (1) by projecting a slide magnified from 24 × 36 mm to 80 × 100 mm with a slide projector equipped with a 150-W lamp and a *f* 2.8-aperture lens (about 55,000 lux on the bitumen). The exposure time ranges from 10 to 15 hours. (2) By contact printing under the Sun (about 350,000 lux) of a positive modern photograph on a transparent film. The exposure time varied from 1.5 to 3 hours. (3) In a camera with a *f* 3.5 aperture lens (similar to Niépce's method for obtaining heliographs with a camera obscura). In this case, our experiments showed that the level of light on the plate was 5,500–11,000 lux. Therefore an exposure time 60–100 hours is required. These times are in agreement with those mentioned by Biot in 1839, who wrote that the Niépce process needed 2 or 3 days under a very intense light to obtain an image from nature in a black box[10]. Thus Niépce could only heliograph static subjects in very intense light. But Niépce's discovery opened the way to his successors.

The exceedingly low sensitivity of bitumen of Judea explains why the different steps of the whole process, such as the preparation of the varnish or its dissolution after exposure, can be carried out under room light without damage. It seems that the determination of the exposure time is the main obstacle preventing the reproduction of Niépce's process. Some historians have eronously concluded that the exposure time was 8 hours for a landscape illuminated by a bright Sun[9].

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Stripes on a pterosaur wing

SIR—D. Martill and D. Unwin in their recent Letter to *Nature*[1] described a pterodactyl fossil in which details of the soft tissues of the wing membrane were preserved. Scanning electron microscopy revealed an epidermal layer on one surface of the specimen, with coarse ridges and finer furrows, below which was a spongy layer said to contain blood vessels. Martill and Unwin interpreted this 'stratum vasculosum', together with an underlying coarsely crystalline layer, as the dermis. Below that was a layer they described as striated muscle, which apparently formed the middle of the original membrane, but the authors did not explain why they thought this layer was muscle. It consisted of polygonal prisms with a maximum diameter of about 40 μm, on which Martill and Unwin said that "transverse striae and nuclei" were visible at high magnification. They did not illustrate these striae, or give any details. The features seen in the electron micrographs would presumably be annular ridges or grooves around the circumference of the prisms.

There are examples of fossils in which very fine details of the surface of soft tissues have been preserved, apparently by the deposition of a calcite 'mould' of the surface, before the tissues decayed[2,3]. This process preserves only surface relief, and it is unlikely that intracellular structures such as muscle striae (sarcomeres) could be preserved in this way. The sarcomeres of fresh striated muscles have a solid appearance under the light microscope, but are not accompanied by annular corrugations on the surface of the fibre. It is conceivable that the sarcolemma might be deformed into annular wrinkles in a strongly contracted muscle fibre, but striae formed in this way would not represent sarcomeres. For a surface mould to be formed that would show such features, the muscle fibres would in any case have to remain in tetanic contraction for some time after death, which seems unlikely. On the other hand, passive elastic fibres would contract when the animal died. Elastic fibres, if they were enclosed in a relatively inextensible sheath, might well exhibit a surface pattern like that reported by Martill and Unwin in their paper.

I reviewed evidence[2] indicating that the ridges on the wing surfaces of certain pterosaur fossils are not stiffening fibres, as had been previously thought, but are wrinkles in the surface of a soft membrane, caused by the contraction of robust (but unseen) internal elastic fibres. These hypothetical elastic fibres would have to be orientated transversely to the wrinkles, not parallel to them as shown by Martill and Unwin in their Fig. 3*c*. Martill and Unwin's observations could be interpreted as the first direct evidence for the existence of these fibres.

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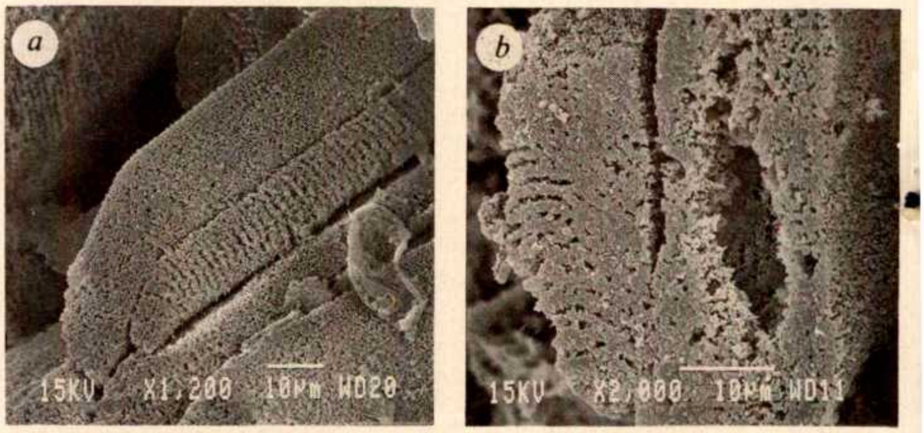
MARTILL *ET AL.* REPLY—Pennycuick suggests that we may have misinterpreted the polygonal fibres in our exceptionally well-preserved pterosaur wing membrane from the Santana Formation (Lower Cretaceous) of Brazil. We concluded that the fibres represent striated muscle replaced by cryptocrystalline calcium phosphate. Pennycuick believes that the fibres represent his hypothetical 'elastic' fibres[2], which he suggested as a possible mechanism for contraction of the flight membrane on the basis of impressions in fine-grained sediments. We here review our evidence for interpreting these fibres as striated muscle and report briefly on our new studies of this tissue.



a, Striated muscle fibres from pterosaur wing membrane from the Santana Formation, Brazil. *b*, Oblique section through individual fibre demonstrating continuity of surface banding through body of fibre. We interpret the banding as fossil sarcomeres.

The most common soft tissues found in the Santana Formation are striated muscle and gill lamellae of elopomorph fish[4]. Here, the muscle fibres are found in their original myomeres, and there is no doubt that they represent fossil striated muscle fibres. They even show sarcolemic membranes, cell nuclei, myoseptal membranes and sarcomeres[5]. Preservation is by replacement with calcium phosphate as demonstrated by powder X ray diffractometry. The fibres we found[1] resemble very closely the muscle fibres found in the fish. Furthermore, pterosaur fibres display a regular banding seen on the surface.

Pennycuick suggests that striated muscle would not be preserved as a series of ridges on the surface of a fibre. Indeed in this respect he is correct. Our striated muscle fibres are not preserved as a series of ridges. Instead, they are preserved as a series of stacked 'disks' along the length of each fibre (*a* in the figure). We have carefully made a new section through the preserved muscle fibres such that several individual fibres have been cut obliquely, showing that the disks are present through the fibre, as would be expected if sarcomeres were preserved (*b* in the figure).

Pennycuick's surprise at the exceptional preservation of our material could be because the material he examined was represented by external moulds in fine-grained micrite. This style of preservation can only reveal details of external surfaces, the resolution of which is governed by the grain size of the sediment host. Our material, on the other hand, is a replacement, in three dimensions, by crystallites with an average length of 300 nanometres. The resolution achieved by this style of preservation allows the identification and accurate observation of three-dimensional intracellular structures. We are confident that the fibres are those of striated muscle tissue.

But our new section shows some structures between the muscle fibres and the stratum spongiosum (labelled sparry calcite and kerogen in Fig. 3*a* of ref. 1), as yet unidentified, that could be Pennycuick's hypothetical elastic fibres or Wellnhofer's stiffening rods[6]. In either case it appears that the pterosaur wing membrane is a highly complex organ, not just a piece of stiffened skin.

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■ Martill's data on fish, referred to above, are published on page 171.

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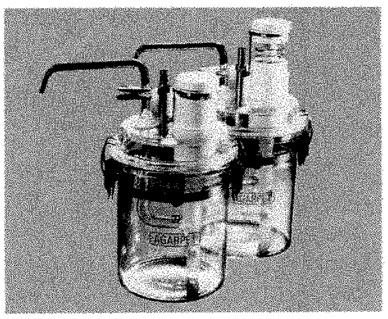

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Matters of life and death

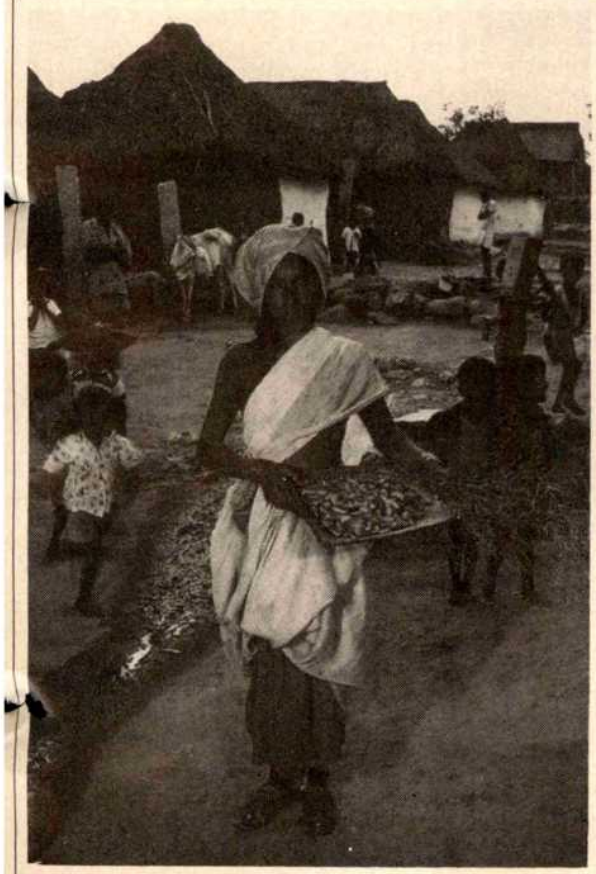
F. E. G. Cox

Lives at Risk: Public Health in Nineteenth-Century Egypt. By LaVerne Kuhnke. *University of California Press: 1990. Pp. 233. $40.*

NINETEENTH-century Egypt was not an idyllic place. Dysentery, cholera and Egyptian chlorosis were endemic in the villages and sewage often contaminated the water supplies of Alexandria and Cairo. Malnutrition was everywhere and epidemics of smallpox and plague had ravaged the whole country by the turn of the century. The medical expertise that did exist was largely based on mediaeval Arab medicine but practised, not by professionals of earlier centuries, but by hakims whose training consisted of copying the standard texts in order to memorize them. It is against this background that LaVerne Kuhnke traces the development of public health care in Egypt, with numerous side glances at the implications for health in the developing countries today.

Changes in Egypt began when Muhammad Ali, who had seized power as governor in 1805, formed a conscript army and, anxious about its health, introduced Western medical institutions and technologies which, Kuhnke argues, were not always appropriate to the needs of the country. In 1827, Ali established a Western medical school modelled on those in France and staffed largely by Italians. Twelve of the first batch of graduates continued their training in Paris in 1832 and five returned to form the nucleus of the school. Their successors made significant contributions to medicine, the most notable being Griesenger, who found that Egyptian chlorosis was caused by hookworms, and Theodor Bilharz, who discovered *Schistosoma haematobium*, the causative agent of the disease that now bears his name, bilharzia. In 1832, a medical school for the training of hakimas (women licensed to practice medicine) was founded, a remarkable achievement in a Muslim society. By 1840, responsibility for health care had also passed to headmasters in schools, with severe penalties for negligence. Thus, by the middle of the century, the elements of public health care had been well established and epidemics were no longer allowed to run unabated.

Epidemics of cholera in 1831 and plague in 1834–36 were countered by rigorous quarantine regimes. An international quarantine board had been set up some years earlier on advice given by the French council of health, but the 1831 cholera epidemic caught it unprepared. By 1848, as a result of a combination of quarantine measures and the implementation of British sanitary reforms, the next epidemic, although severe, was more easily contained. Plague also ceased to be a serious problem and after 1844 it disappeared from Egypt for 55 years. Vaccination against smallpox was both efficient and effective and between 1837 and 1840, 61,000 childen had been vaccinated in Cairo alone although there was strong opposition and non-compliance in many villages.



More than half of India's land surface is cropland — the soils are fertile and robust, and rainfall adequate. These factors have combined to make India the most densely populated large nation on Earth; 80 per cent of its inhabitants live directly from the land or in the service of small rural communities. From *Man on Earth*, just published in Penguin paperback, by John Reader. First published by Collins in 1988, the volume records the author's attempts to understand humanity. Reader looks at culture from a functional point of view, looking at how adaptation and regulation have promoted the continuing existence of people in a wide variety of environments throughout the world. Illustrated with the author's own superb photographs, price is £9.99.

The evidence presented seems to suggest that the control schemes used actually worked, but Kuhnke questions whether the Western ideas on which they were based were appropriate for Egypt. This is the weakest part of the book and detracts from an otherwise scholarly and dispassionate work. The author perceives an apparent distinction between "authentic medicine", equated with curative medicine, and community and public health concerns, concluding with these words: "As long as the West upholds urban hospital-based curative medicine for the individual as the ideal for 'health care', the lives of rural Egyptians and many others may continue at risk." This is an entirely false picture of how biomedical science interacts with the needs of the developing countries (see, for example, *Biomedical Science and the Third World*, edited by Barry Bloom and Tony Cerami, *Annals of the New York Academy of Sciences*, Vol. 569, 1989). Tropical medicine, and the World Health Organization in particular, is as much concerned with prevention as with cure and the two run together. It is a pity that Kuhnke did not consider bilharzia, as this is a disease that has always been associated with Egypt and from which much can be learned. The conflict for public health strategy is between the needs of water for irrigation and the consequent inevitable spread of the snail vectors. Control of bilharzia is based both on snail control and on chemotherapy, which is essential to reduce contamination of snail-infested waters.

History does repeat itself, and the resistance of some villagers to vaccination and their failure to return for booster doses is still a problem today. It is also interesting that seven of the first twelve graduates of the medical school chose to remain in Paris, and the pile of examination scripts now on my desk reminds me that twentieth-century students also learn large parts of textbooks by heart. ☐

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In the balance

Edward Abraham

Miracle Cure. The Story of Antibiotics. By Milton Wainwright. *Blackwell: 1990. Pp.196. £16.95.*

MILTON Wainwright has written a readable pot-pourri. Much of it is concerned with the assessment of credit for the discovery of the clinical value of penicillin and streptomycin, but the author strays into other areas, including a description of some bizarre procedures that have previously been claimed to cure bacterial infections and the fiasco of the antibiotic patulin and the common cold.

The author, who is in the Microbiology Department at the University of Sheffield, states that he has "attempted to redress recent attempts to downgrade the role played by Sir Alexander Fleming in the discovery of this most famous antibiotic". Some may wonder whether this is necessary, for Fleming's name has been embedded in the public mind, while Florey's and Chain's have not. Yet Fleming was not elected to the fellowship of the Royal Society until 1943, 20 years after he had first been proposed by Almroth Wright. Wainwright suggests that this should carry little weight with "anyone who is familiar with the routes by which one can succeed to this august body". The precise implication of his phrase is not clear. But great trouble was taken to ensure that Fleming's candidature was not adversely affected by publicity for the findings in Oxford in 1940 and 1941. And it should be remembered that the Royal Society is concerned with science, not merely with medicine.

Fleming's chance discovery of penicillin has never been seriously disputed, nor has his early belief that it might be clinically useful for local application to infected wounds. The vital discovery at Oxford, which receives little emphasis in the book, was that even exceedingly impure penicillin could cure generalized and life-threatening infections when introduced into the blood stream. It was this that gave penicillin its outstanding medical importance. All that is arguable is whether Fleming could have demonstrated this property in mice if he had thought of trying to do so.

This book contains several interesting anecdotes which may not be widely known. Appropriately, it describes in some detail the striking success in Sheffield of Dr C. G. Paine, whose penicillin-containing culture filtrates from Fleming's *Penicillium* were used for local treatment of eye infections in about 1930. Paine never published these results, but later he sent notes on the cases to Florey. These notes (not mentioned by Wainwright) were reproduced in 1949 in *Antibiotics* by Florey *et al.*.

Wainwright provides simplistic and slightly misleading accounts of the chemistry of penicillin and the semi-synthetic penicillins and cephalosporins. Because all these compounds have a four-membered β-lactam ring it might have been more appropriate to have placed them in the same section of the book.

The discovery of streptomycin was reported in 1944 by Schatz, Bugie and Waksman at Rutgers University; this antibiotic was later found to be effective in the treatment of tuberculosis. This book describes how all was well between Waksman and Schatz until Schatz discovered that Waksman was receiving money from Merck and began a lawsuit for a share of royalties. The case ended with a partial victory for Schatz. However, when a Nobel prize was awarded to Waksman alone, Schatz could not bring himself to remain inactive but, predictably, he gained nothing from his activity. Wainwright states that he has tried to redress an historical imbalance, "this time in favour of Albert Schatz". Whether the balance needed redress is at least open to question. Perhaps Waksman was ungenerous to Schatz and too secretive. But it seems that Schatz failed to appreciate that he was a research assistant to a man who had opened a wide field of investigation that had already led to the discovery of streptothricin. What is beyond doubt, however, is that misjudgements by Waksman and Schatz brought them both a great deal of misery.

The Hutton picture Company

Fleming — "embedded in the public mind".

This book is well produced. But some readers would find a more detailed index more useful and others might appreciate the addition of a few primary sources to the author's suggestions for further reading — to redress its balance. ☐

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A way through the jungle

Colin Gough

Physics of High-T_c Superconductors. By J. C. Phillips. *Academic: 1990. Pp.393. $55, £36.*

ALMOST four years after Bednorz and Muller's Nobel prizewinning discovery of superconductivity in the layered cuprate compounds, the unexpectedly high transition temperatures of these materials remains unexplained. It is frequently claimed, with some justification, that there are as many theories for the layered cuprate high-temperature (or HTC) superconductivity as there are groups working on the problem. Keeping abreast of the literature remains a daunting task for even the most experienced researcher.

A book designed to summarize and explain the physics of HTC superconductors is therefore specially welcome, particularly when the writer is J. C. Phillips, who has made many significant contributions to the theory of the electronic and phonon properties of solids including conventional superconductors.

In aiming "to cut a well-marked path [through a literature] as forbidding as the darkest jungle", Phillips has selected a rather special path, carefully chosen to support his view that HTC superconductors are really no different in kind from previously known superconductors. He is sufficiently confident to state "there is no room for doubt that it is lattice instabilities and not magnetism that produce high-T_c superconductivity just as much in cuprates as in intermetallic compounds". Relatively little attention is drawn to other dangerous animals lurking in the jungle in the guise of theoreticians with diametrically opposed views.

The original Bardeen–Cooper–Schrieffer (BCS) model successfully accounted for superconductivity in terms of an indirect attractive electron interaction leading to superconducting pairing via lattice vibrations. This mechanism appears to place an upper limit for superconductivity of around 40 K (−233 °C) — well below the current record for HTC superconductors of 125 K (−148 °C). However, Phillips emphasizes that the layered cuprate HTC superconductors are much more complex structures than the theory was originally developed to describe. Matthias, who spent a lifetime in the quest for higher temperature superconductors, wo[illegible] have claimed that the cuprate superconductors were "tricking nature" into circumventing the apparent boundaries of conventional BCS theory.

To support this thesis, Phillips first presents an extensive background to electron-lattice interactions in old-style superconductors including simple elemental and binary alloy superconductors, binary and ternary compounds, such as the A15 and Chevrel phases. The superconducting cuprates are ternary, quaternary and even more complex chemical compounds — very much more complicated chemical structures than those usually studied by solid-state physicists.

Phillips describes the most important HTC compounds known up to about a year ago and argues the case for superconductivity via low-frequency lattice vibrations or instabilities. Two of the most carefully studied HTC superconductors are $(La,Sr)_2CuO_4$ and $YBa_2Cu_3O_{7-\delta}$, in which the superconducting properties are controlled by strontium and oxygen vacancy dopings, respectively. The relatively facile diffusion of oxygen in the $YBa_2Cu_3O_7$ structure is related to soft lattice correlations involving oxygen vacancies — one of 'nature's tricks' to give superconductivity at 92 K for this compound.

Any successful theory must first account for the anomalous normal state properties. Others have claimed that the remarkably linear dependence of resistivity on temperature common to all the layered HTC compounds reflects an intrinsic property of the electrons in the layered cuprates. Phillips presents a more mundane explanation in terms of electron scattering by the same low-frequency lattice vibrations that ultimately lead to superconductivity.

Who is right remains an open question. On balance, more recent measurements would tend to favour the former view. But it would not surprise me if Phillips turns out to have the last word. The ability of theoretical physicists to adapt their theories to fit inconvenient experimental facts is legendary. Experimentalists can also all too easily become locked into believing the current fashionable theory, with no better justification than other competing but less fashionable theories. At this stage in the development of our understanding of HTC superconductors it would be wise to keep an open mind.

Provided the reader recognizes that this book is not an introductory text but is a research monograph intended to argue a particular point of view, it can be strongly recommended — particularly to those with a solid-state physics background. ☐

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- Recently published by Plenum is *Oxygen Disorder Effects in High* Tc *Superconductors*. Edited by J. L. Morán-López and I. K. Schuller, the volume contains the proceedings of the conference held at the International Centre for Theoretical Physics in Trieste in April 1989. Price is $59.50. ☐

Object lesson

Richard Davenport-Hines

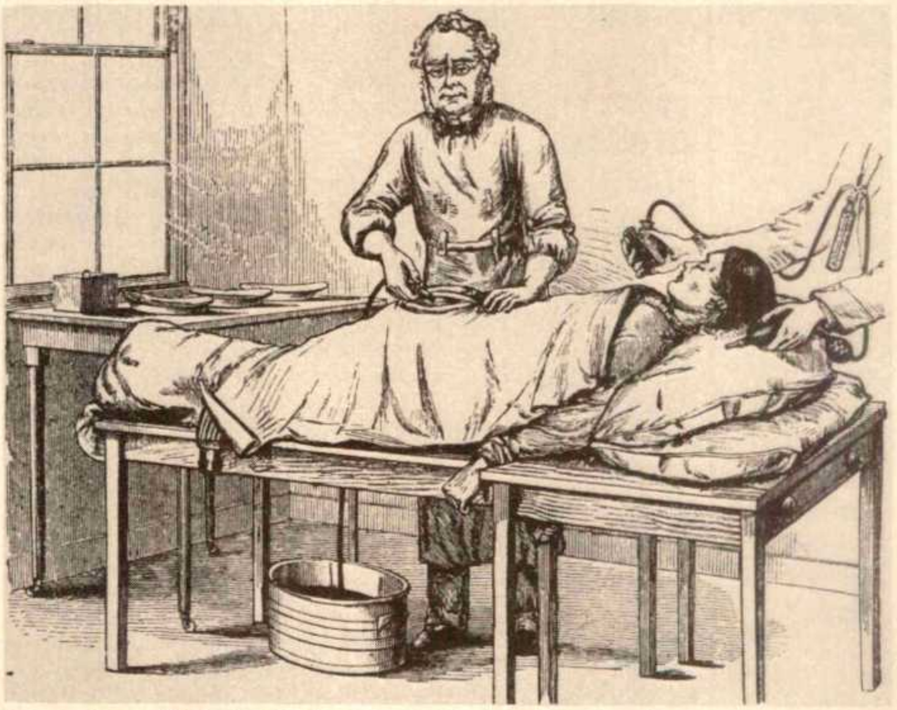
The Science of Woman: Gynaecology and Gender in England 1800–1929. By Ornella Moscucci. *Cambridge University Press: 1990. Pp. 278. £35, $49.50.*

THE history of childbearing is the subject of several recent and excellent books. In addition to Renate Blumenfeld-Kosinski's study of mediaeval caesarean births entitled *Not of Women Born* (Cornell University Press, 1990), there is Valerie Fildes' *Wet Nursing* (Blackwell, 1988) which traces changing attitudes to breastfeeding from antiquity to the present day, and *The Politics of Maternity Care* (Oxford University Press, 1990), in which Jo Garcia and her colleagues have collected a range of historical essays on services for child-bearing women in twentieth-century Britain. This burgeoning literature is now joined by Ornella Moscucci's study of the influence of gynaecological ideas and practice on scientific notions of gender.

Moscucci focuses on London teaching hospitals, but the implications of her study are wider. She questions science's autonomy from society, and challenges the neutrality of its mediations, arguing that the social relations between men and women in the nineteenth and twentieth centuries were reflected in medical notions about masculinity and femininity. She shows deftly and authoritatively how the personal and professional circumstances of British gynaecologists fashioned both the ruling principles of gynaecology and affected the treatment that was offered. She analyses how the wish for professional exclusiveness turned gynaecological practices into weapons in the battle for professional hegemony — for example, how the "envy, hatred and malice" which existed between surgeons and obstetric physicians, especially in the 1884–1929, was fired by controversy over the issue of ovariotomies. The ill-effects on patients when hospital hierarchies become too ruthless in their self-perpetuation makes sad reading.

The scientific study of femininity in nineteenth-century western Europe began as a genre combining scientific findings with literary quotations and philosophical speculation. It fastened onto the processes of puberty, menstruation, childbirth and menopause as indications of an essential animality in women, with the result that medical writings increasingly depicted women as unstable, illogical and the victims of their own bodily emergencies. The subordination of women was enforced by medical teachings, while female sexuality was represented as a threat. An article in the *Obstretical Journal of Great Britain* in 1874 typically described women as the "reproductive servants of their race", and a decade later a leading obstretical textbook depicted the ovary as "ever struggling for supremacy, and cannot long be kept in subjection". The physiology and pathology of the female sexual system were represented as defining women's personalities. As a physician wrote in 1916, "it is because of all the internal secretions that woman is what she is".

Moscucci is far from representing all women as the passive recipients of male medical care, and shows how many people



Ann Ronan Picture Library

Thomas Spencer Wells, the British gynaecologist who perfected ovariotomy.

esisted inappropriate treatment by men. Her book offers many interesting sidelights on issues of female surgery, on the practice of antisepsis, on the use or abuse of the speculum, and on craniotomy as a technique in childbirth. She recognizes the complexities of her subject, and neither adulates physicians as infallible scientific titans nor lapses into the crude petulance of other medical historians who tend to present all patients as the victims of despotic medical authority.

This monograph is the latest volume in the Cambridge History of Medicine series, which is reliably distinguished by scrupulous research and fine historical judgement. (Moscucci is writing the anniversary history of Britain's Royal College of Obstreticians and Gynaecologists.) *The Science of Woman* maintains the quality of the Cambridge series, and although it has too much of the tautness of a PhD dissertation, it is imaginative and well-written. Moscucci's insights have been enriched by her reading in modern feminist historiography, but she dissents from some of its orthodoxies in passages which are notably reflective and honourable. The integrity of her ideas and the honesty of her doubts are as impressive as the range and power of her research. ☐

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Wearing sheep's clothing

Peter Coles

Trois Pattes pour un Canard (Souvenirs d'un biologist). By Etienne Wolff. *Editions de la Fondation Singer-Polignac, Paris: 1990. Pp. 201. FF130.*

A CELEBRATED experimental embryologist, former director of the respected Collège de France and member of the Académie Française, Etienne Wolff has been nicknamed "the monster man". Although he dislikes — and rather resents — the title, it was inevitable. His most well-known work has been to produce live monsters in the laboratory — cyclopean chicks, a duck with a third leg where its tail should be, birds with heads sprouting from their intestines and others, equally grotesque. Commonplace in contemporary embryology, teratology was an unfashionable and little explored domain in the 1920s. Now, at the age of 86, Wolff has written a set of 'biographical essays' and a short note on his research to allow us a peek behind the off-putting nickname.

The image Wolff gives us of himself is sometimes candid, but the 'essays' remain mostly rather superficial and arbitrary. The overall theme is autobiographical, but the essays do not fall into chronological order.

Sandwiched in between "childhood" and "orientation of my studies and career", for example, is a chapter on "Judaism and anti-semitism". After a lifeless and rather banal description of his trips to Japan, the United States and the Soviet Union, there is a chapter on "animals and me", where he defends animal experimentation, saying that real cruelty is found in the commercial exploitation of animals, not in the laboratory. But accusations of cruelty associated with his research obviously weigh heavily, despite his light-hearted self-mockery, since almost too much space is given to show he is an animal lover. While the front cover shows a disturbing picture of a three-legged duck, the back cover has a picture of the author cuddling his own cat, "Grouillot".

At the beginning we are treated to anecdotes about Wolff's childhood and his early education, including descriptions of a Paris with steam trams and open-topped buses. Yet these do not really come alive, partly because the manuscript does not appear to have been edited, leaving a cumbersome and repetitive prose. Then there is a sketch of the meandering university studies that led from a degree in letters to a research post — rare at the time — at the Institute of Embryology of the University of Strasbourg. Here began his initiation to embryology and agement to explore the largel area of experimental teratolog

Early in his career, Wolff remarkable discoveries. He fo hormones, not proteins, det sex of chicks and that gen changed in mid-course of de He also found that very specifi an embryo at a given date in h consistently produces a given sity'. With the outbreak of career was suddenly interrup 1940 he was taken prisoner. H next five years in prisoner-of-w Austria and Germany whe handful of fellow academics, 'mini-university', giving lectur soldiers. His two books, "Sex and "The Science of Monsters" after the war, were both writte in Germany. Returning to after the liberation, he built u laboratory with a staff of 50. T when he was awarded a chair at de France in Paris, where his la the Bois de Vincennes becam the Mecca of Embryology.

Wolff says very little about h in this book. In a mere 50 page 200 he describes how he coul regularly and systematically, 'double' monsters, cyclops an cephalic chicks (with heads breasts). Other work include organ cultures *in vitro* and ch sex of chicks. But although he dispel the idea that he is a ki Frankenstein" — most of hi appear spontaneously in natur not told why this work is impor

Much space is devoted to Wolff's teachers, to friends m prisoner-of-war camps, and t eagues, yet even these some depth. Similarly, for a Jew himself in the hands of the Na who also experienced the antis Pétainist France — Wolff's c antisemitism is surprisingly and even comes across as an a resents the word 'Jew', prefer called an Israelite — ironically terms used by Arab antisemites

All in all, this book will plea friends, colleagues and fellow cians. But it does little to "sell" to a broader public and gives a d ingly reluctant, or over-modes of the life of one of the leading century French biologists. O help but come away with the im a brilliant, but very sensitive while having enjoyed the highes from his peers, has never com with the abuse from his detracto

Peter Coles, 3 rue Simon Le Fr Paris, France, is a freelance jo Nature's Paris correspondent.

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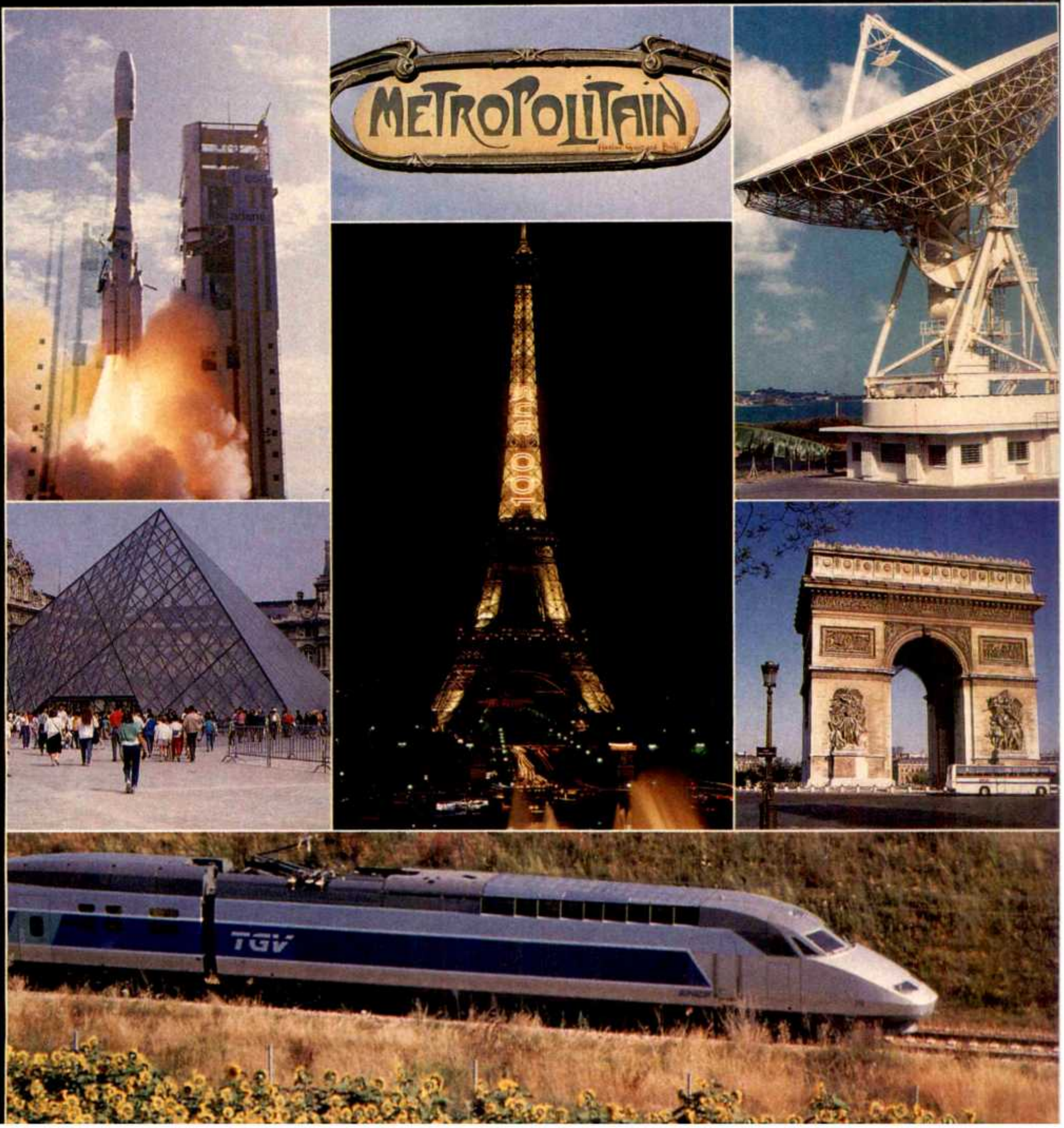

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The resurgence of science in France

The Socialist government of France has indeed done wonders for the health and achievement of French research, but there is a raft of problems — some of them long-standing — still to be resolved.

FRANCE, once the most conservative country in Europe, has become a land of technology buffs. People reserve seats on the high-speed trains whizzing everywhere (as well as theatre tickets) by Minitel, the domestic video-tex machines that have swept the country — and which could not have functioned if the telephone system had not been superbly modernized in just ten years. France has also provided the impetus for the creation of the only civil aircraft manufacturer outside the United States capable of selling civil aircraft to the world's airlines. Now it is seeking to drag its European partners into space. What has brought about this transformation? Do its roots lie in the election of François Mitterrand's Socialist government in 1981, and in its deliberate and daring programme to reinvigorate science and technology?

Neither the nature of the transformation nor the importance of its apparent cause should be exaggerated. France remains the Western European state most apt (and able) to claim to be the custodian of European civilization. (The cuisine is merely a by-product.) And technology has not ruined the delectable appearance of France, only some of it. (Too many city mayors have commemorated themselves with ungainly blocks of concrete in mediaeval settings.) Civilization does not, of course, equate with civility, but with respect for the law (including the Revolution's slogan), for intellectual discourse and the importance of the imagination.

The impetus for change in the 1980s is also a prolongation of earlier tendencies. How could it be otherwise when even provincial street names commemorate Descartes, d'Alambert, Pasteur, the Curies (both of them) and the like? The modernization of French science and technology began with the Gaullist government of 1958 and with the creation of the Délégué Général de la Recherche Scientifique et Technique (DGRST), which rapidly transformed itself into a kind of unestablished ministry of science.

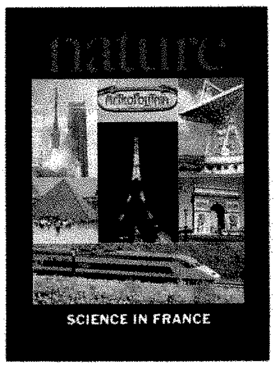
Much of the Gaullist government's support for science and technology sprang from its preoccupation with the military defence of France, but that is when modernization began in earnest. The decision in 1981 that the budget for civil research should increase by more than 16 per cent a year was but the arithmetical consequence of the decision that civil research and development should be 2.5 per cent of Gross Domestic Product by 1985. The name to conjure with is that of Jean-Pierre Chevènement, Mitterrand's first minister of research and industry, now defence minister in Mitterrand's second Socialist government.

The timing of this survey of French science is deliberate: it may not be too soon to estimate the outcome of what an earlier survey (*Nature* **296**, 285; 1982) called "the grand experiment". The outcome should be of interest not only in France but elsewhere, in Britain for example, where governments have taken a more jaundiced view of the benefits of public support for research. But the method of this survey differs from that of earlier exercises of this kind. What follows is not an account of the well-known centres of excellence in France, but is based on conversations (often informal) with working scientists in France and elsewhere, as well as on documents in the public domain.

The good news is quickly stated. In the past eight years, there has been a quite remarkable deepening and broadening of basic research in France. Everybody knows of research groups in France with long-standing international reputations — the mathematicians of Nancy and Marseille, particle physicists at Orsay, solid-state physicists at Grenoble and Strasbourg, molecular biologists at Paris, the Pasteur institutes and Strasbourg, genome mappers and geophysicists at Paris, chemists almost everywhere. The psychological effect of the grand experiment is that all research groups can now realistically aspire to excellence of that kind. And most seem to have that ambition, as well as the enthusiasm that may overcome the usual bureaucratic obstacles.

Two other features of the transformation stand out. First, French researchers seem finally to have buried chauvinism in the search for international collaboration. A united Europe, in particular, is now, not tomorrow. Second, more by accident than design, the French government has engineered a bridge between basic and industrial research that is well designed to keep the technological revolution bubbling away in France.

That is the good news. Some of the bad news will be found on the pages following. □



Science in France

This survey has been written by John Maddox, the Editor of Nature, and Peter Coles, Paris correspondent, both of whom are grateful to researchers and public officials in France for assistance often given at short notice. Cover photos: Feature-Pix/CNES-CSG/SNCF-Lafontant.

Flies in the ointment

French research has been a great success, but there are structural weaknesses in the system that cry out for attention. French science is in better shape now than ever, so why is there so much talk of emergency, even crisis? One of the strangest features of France's distinctively explicit way of managing research — every major item of public expenditure is explicitly analysed in advance — is the recognition that rationality can often be the harbinger of muddle.

What follows is a stark list of those features of the research enterprise in France that cry out for attention. To the extent that they amount to complaints against the present system, they represent either the opinions of working scientists or are administrative faults acknowledged as such by the managers of the system.

It is important for an understanding of what should now be done that most working scientists are employed directly by the national government. This applies both to those who work for the *grandes organismes* of research (organizations such as CNRS), who acquired the status of civil servants in the early 1980s, and to those who are university teachers. Only during the past decade has the number of technical people working for industrial companies shown signs of substantial growth.

It is also relevant that the national universities are part of a unified national system, lacking the autonomy with which liberal opinion would endow them. Students are admitted on the strength of their performance in the school-leaving examinations, and the power of the universities to impose further selective tests is limited. (But several universities have been seeking to do just that in the admission season now under way, provoking fierce protests.) And the most sought-after degrees are those whose award is validated nationally, on the basis of an approved curriculum and examination.

Against that background, this is where the problems lie:

■ **Universities**. That the universities are in danger of being overwhelmed by their students is generally acknowledged. Wedded to open access by the memory of the troubles of 1968, the government is making a virtue of necessity (see page 133). One danger is that only the most ingenious and tenacious students will acquire a good education in the circumstances that now obtain. Another is that the assumption that research and scholarship are a crucial part of academic work will become even more of a dead letter than it is already.

The government acknowledges that there are problems, and is doing what it can to solve them (see page 134). Principles of good management would dictate that there should be a greater devolution of autonomy to the universities; whether the device of the four-year contract between the education ministry and individual universities will have that effect will, in the end, depend on whether the government can sweeten its contracts with extra money.

Meanwhile, the plan to provide extra research funds to academics through the agency of a committee located administratively within the ministry of education seems an invitation to nepotism and soft judgement which have given French universities a bad name in the past. If the universities are to win genuine autonomy (a necessary condition if they are to ease the centre's financial burden by attracting funds from regional governments), then *France will need its own version of a national grant-making body distinct both from the ministry of education and from established organizations such as CNRS.* Why not begin now, before the suspicion becomes entrenched that the ministry of national education, reconciled to a measure of university autonomy, is planning to control them in the long term by research grants?

■ **Student support**. The legend of impoverished students eking out life in Paris garrets is too affectionately regarded to be exorcised quickly, but French inhumanity towards graduate students needs to be abated, especially now that the ministry of national education has acknowledged that it will not be able to teach students in future decades without more university teachers qualified at the PhD level.

The standard route to academic life is through the qualification of the one-year DEA (Diplôme d'Etudes Approfondies), involving (for researchers) laboratory work. Student support through this period, after a first degree, is hazardous enough, but university DEA committees, usually organized on a disciplinary basis, become the means by which students are chosen to receive grants to continue with a PhD course.

The numbers of grants seem invariably less than the justifiable demand. Often there will be two or three grants to distribute among 30 reasonably qualified students. Those who administer the system are humiliated that they rarely know in advance how many there will be. They and laboratory directors are embarrassed that so many disappointed students embark on research degrees in great hardship. *If the government wants more "docteurs", why does it not ameliorate the hardships of those who would meet its needs?*

■ **Mobility and employment**. One complaint is that researchers, both established people and graduate students, are much less mobile than is good for the French research enterprise. Another is that France lacks a system of postdoctoral fellowships for keeping new PhDs at work while they cut their professional teeth and decide, if successful, what fields they will follow. A third is that scientists joining the research organizations are underpaid, and have to wait too long for promotion.

All three problems are linked, and require a common solution. The habit of immobility is engendered by the system requiring that students seeking a research degree must first acquire a DEA qualification, which entails close relationships with research laboratories at which, as likely as not, success will bring them their first research job.

The lack of postdoctoral fellowships *for French researchers within France* means that the transfer of new techniques from one place to another is impeded, while able and established people cannot quickly acquire the help they need to exploit new ideas. (With a few exceptions, even the most influential research groups in France are much smaller than their opposite numbers elsewhere.)

The solution is so obvious as to be breathtaking. *Why not convert established posts in the research organizations such as CNRS into three-year postdoctoral fellowships tenable wherever in France the holder chooses?*

The result would be that able people would get their first taste of independent research at the laboratories that seem to suit them best, that successful research groups would be better able to recruit the help needed to exploit new opportunities and that successful university researchers would be put on a more equal footing with those at the public laboratories.

This is the simplest change of the system there could be. People already on the first rung of the ladder of public service research would not be displaced or otherwise inconvenienced. There would be no significant extra cost — it would be necessary to endow host laboratories with research expenses, but they arise now when new people start work at the laboratories. And it would be possible for promising research groups successful in the competition for people to become competitively strong in a reasonably short time. One of the glaring defects of the present system is that the best groups are consistently understaffed and overworked.

The management of the public laboratories would no doubt be discommoded by a failure to recruit sufficient postdoctoral help to keep their programmes running, but the other side of that coin is that they would be better able to tell who should afterwards be taken on permanently.

■ **Bureaucracy**. The temptation to suppose that a national government modelled by Napoleon is rife with bureaucracy should be resisted. In reality, the government is both accessible and informal. Ministers consult interested parties, and seem to take good advice wherever they can find it. And while the law is the law, most regulations are decrees, which can be relatively easily amended.

It is nevertheless irksome, and a considerable impediment to the welfare of the research enterprise, that questions of money and of the appointment of people should habitually require reference to the centre. That people should have to travel to Paris to intercede with officials over the rejection of applications for small amounts of money is similarly humiliating and a waste of time whose value is now appreciated in France more acutely than ever. *Now that devolution is under way* (see page 131) *cannot the Paris lawyers find a legal mechanism for behaving as if the government's researchers were honest people?*

There is a particular problem over the acquisition of special skills, computer programming for example. Few such people will work in government research because they can do much better elsewhere, but laboratories cannot employ them even on short-term contracts for fear of creating illicit government positions. And there is not enough money to purchase all the software needed from outside contractors, so that far too many researchers double up as their own programmers. *Even in France, there should be a solution.*

- **Money**. France compels admiration for the changes brought about in the past decade and more in the economy at large, not simply in research. It is now a much more prosperous country, even if there are constant envious looks across the border to the East — at West Germany. *Yet the government may have underestimated the true cost of its ambitions for research.*

The calculation is that no great harm may be done if salaries in the public service are so low that people can easily be tempted away to industry, but that will not be true for ever. Despite the success of schemes for adding to research spending from other sources, there is ample evidence that many able people could do much more were it not for the accentuating lack of operating costs. That, on balance, is not simply a waste of people but is potentially destructive of what must now be the most encouraging feature of French research — the generally growing general conviction that every able person can make his or her mark. ☐

POLITICS AND SCIENCE

Chevènement's legacy to the 90s

WAS Jean-Pierre Chevènement the cause of the transformation of French science in the past decade, or was he himself the product of a process already under way?

The question is still much debated, for which reason it is important that the tense in which it is asked should not be taken as a sign that Chevènement belongs to history or, worse, is dead. As minister of defence in the French government, he spent several days of last month on an aircraft carrier in the Atlantic. One day last week, with three other ministers, he was present at a passing-out parade at the national police college.

Yet there has never been a period in the recent history of the administration of French science quite like the year beginning with François Mitterrand's election as president on 10 May 1981. During the election, the Socialists had made no secret of their ambitions for research, but the reality of Chevènement's plans for increased spending were nevertheless a surprise for many people. The research budget for 1982 turned out to be 25 per cent greater than that for 1981.

This daring leap forward had been planned in advance by a working group under the chairmanship of Professor François Gros of the Pasteur Institute. Mitterrand spelled out his policy for research at a meeting at the Luxembourg palace on the eve of the election. In the international competition for discovery, will France be at the front or the back? We may be poor in natural resources, but we are rich in grey matter. That was the point at which Mitterrand promised that research and development expenditure would be increased to 2.5 per cent of the gross domestic product by 1985 — a promise subsequently written into law.

Chevènement's energy and enthusiasm seem to have been crucial. Gros has told how, soon after the election, he had visited Chevènement to explain that, whatever else was done, "the first essential is to change the relations between people" in research; his fear was that the imposition of new structures and tasks on a research community already disillusioned by neglect would bring further alienation. Why, he asked, not organize a great national colloquium at which these issues could be argued out?

The notion was not entirely novel; in 1956, the Mendes-France government had organized a colloquim at Caen. But neither Gros nor anybody else appears to have anticipated the energy that Chevènement would put into the project, which fitted with his own political position in a syndicalist faction of the Socialist Party.

Between October 1981 and the end of the year, no fewer than 31 three-day meetings were organised throughout France, culminating in a four-day meeting in Paris in mid-January 1982. It is estimated that some 25,000 people took part in the regional meetings.

The organization of this gigantic consultation was carried out by a committee under François Gros, with Dr Philippe Lazar (then the president of the scientific council of INSERM, now its director-general) as vice-president and rapporteur. Even now, Lazar vividly recalls how, after nearly a year's intensive work on the organization of the colloquia, he had been looking forward to a vacation. Instead, Chevènement asked him to move to INSERM right away.

Among others, one of the emergent themes was the importance of research as a means of bridging the economic gap between rich and poor countries, now embodied in the great emphasis in French research on the problems of tropical agriculture. But the overarching theme was that science belongs to the people and is a part of the general culture. ☐

Colloquia galore

JEAN-Pierre Chevènement's most memorable achievement may have been his decision to hold a series of regional colloquia (*assizes*) at which members of the scientific community could voice their aspirations and discontents. Certainly, he has many imitators.

Beginning this year, the ministry of research and technology plans a series of such colloquia dealing with particular fields of research. The first of them, this January, launched "cogniscience" on the psychology and neurobiology communities. Earlier this month at Strasbourg, there was a similar meeting on planet Earth.

Science minister in the early 80s, Jean-Pierre Chevènement.

The objectives are several — to improve coordination in continuing fields of study, to plan how to exploit emerging areas (order and chaos, for example) and to explore the relations between academic studies and their application.

But the Ministry of National Education has now joined in, with a colloquiun at the Sorbonne last month which gave the minister, M. Lionel Jospin, the opportunity to repeat Allègre's slogan "Diversity means equality" (see page 133). ☐

UBLICANISM

The heat of competition

One should not forget that the republican spirit is alive and well in France, more than 200 years after the French Revolution. The grandest people are called "monsieur", as in "Monsieur le Président". And some of the grandest people every day shake hands with those who sit outside their doors, letting through those with appointments and diverting others elsewhere.

Another practical consequence is the institution of the *concours* — the system of competitive examinations by which people compete for places in the public service (which includes student places at the *grandes écoles*, whose *concours* are anonymous — see page 135) throughout France. The objective is to banish nepotism. One Briton working at an INSERM laboratory fulsomely admires the system, which he says should be adopted everywhere.

But there are obvious drawbacks for the management of laboratories. In CNRS, for example, the competition for places (held twice a year) means that particular laboratories cannot be sure that promising PhDs whom they would wish to fill particular slots will succeed in the national competition for places at the first, or second or even umpteenth attempt. Successful directors must have a supply of soft money (perhaps from industrial contracts) up their sleeves.

Promotion is also determined by *concours* — and the chance of success by the low ratio of vacancies to applicants. The obstacles to promotion to the status of *directeur de recherche* (group leader) is one of the chief discontents of young research workers in the public service.

The tendency towards centralism in France, everywhere apparent, also has other origins, mostly Napoleonic. The disadvantages of centralism have traditionally been freely acknowledged, but people have then shrugged their shoulders as if to say that little can be done about them. One of the achievements of the second Mitterrand presidency is that the cosy conventions of centralism are being eroded. The calculation seems to be that French economic growth will be faster if devolution gives regions an influence on their own welfare. That seems to be correct in the case of at least one region in the southwest (see page 131).

But decentralization is being worked out in a centralist fashion; there are the same rules for all the regions, while the devolution of responsibility for education, planning and economic development is far from complete. But in the long run, regional involvement in educational matters could be crucial for research. ☐

PUBLICATION

"We want to play the game"

Director J. C. Duplessey at the CNRS Radioactive Tracer Laboratory (Institut des Faibles Radioactivités) tells how, when he published his first paper in an English-language US journal in 1971, his professor told him that he had been stupid. "Nobody will read it", he was told. But now the boot is on the other foot. "We want to play the game, and we have learned how to do it."

Where and how to publish is on everybody's mind, but there seems only one answer: in English, and in an international journal. The apparently serious suggestions of a few years ago that people's performance should be determined by a points system, with so many points for a paper in journal X, and so many fewer for a paper in journal Y, have mercifully now been dropped. But there is a general understanding that there is no general case for publishing in French in a French-language journal. Then you will not be read by the people you need to reach.

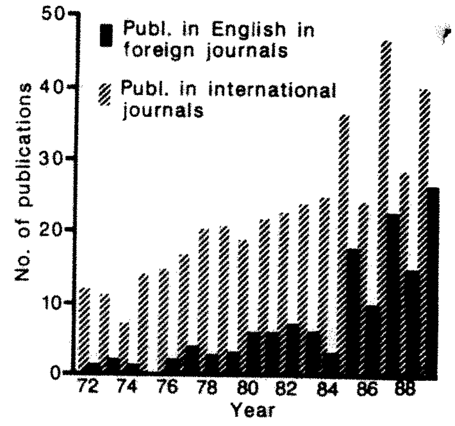
Nor will you be respected by your colleagues and compatriots. The annual reports of many public laboratories include not merely lists of the papers published during the past year, but an analysis of the proportion appearing in foreign-language journals (see, for example, the figure below).

Yet as things are, the change of practice seems to have made little impact on the bibliometric statistics. Recently published figures suggest that between 1981 and 1986 inclusive, the French share of the world's literature fluctuated narrowly and, if anything, that it declined (Martin *et al. Science and Public Policy* **17**, 14–26; February 1990). In 1981, the French share of publications in the US National Science Foundation's file of journals was 5.02 per cent, but had declined to 4.84 per cent in 1984, rising to 4.87 per cent in 1988. (The corresponding figures for the United Kingdom are 8.34 per cent, 8.16 per cent and 8.19 per cent.)

No doubt these figures should be regarded with caution. For one thing, they are inevitably out of date. For another, there is plenty of anecdotal evidence — references to outstanding French papers in well-known journals — to suggest that the benefits of the new attitude towards publication have not yet worked their way through. There is also some weight in the observation of one laboratory director that it was fruitless to go looking for evidence of a resurgence of French science "when we may simply have started publishing in English". But the habit has come to stay.

That the French reputation for chauvinism should be so directly belied is proof of a profound sea-change of opinion, linked with a general appreciation that even the French government's generosity towards research will not enable France to make its own way in a competitive world without external collaboration. So people are also forever on the lookout for opportunities for collaboration, perhaps by the judicious placing of postdoctoral fellows in overseas laboratories, perhaps through collaborations with opposite numbers elsewhere and also through the European Communities.

But compliance does not mean pleasure. For one thing, there are practical difficulties. People whose mother-tongue is French do not write easily in English. (This may be one explanation for the good sprinkling of English-speakers, American or British, in the public laboratories.) More contentiously, it is also asserted that French is intrinsically more capable of clarity — which may be another way of saying the same thing.



In any case, most people seem to hold that while English is now the language in which they must write papers — as it has also become a passable lingua franca of conversation — it will be a matter of great regret if French is altogether abandoned. Philip Lazar, the reflective director-general of INSERM, says he hopes that "the time will never come when no science is written in French".

Nor need it. In contrast with those of the English-speaking world, French researchers still regard the production of books as an honourable occupation. Journals such as *La Recherche*, whose strong suit is high-level popularization, appear to be as much a pleasure to write for as they are to read. And the general popularity of technology as well as the need for interprofessional communication has been a powerful impetus for French technical publishing at all levels.

Yet the clinching sign that the use of English has come to stay must be that the *Comptes Rendues de l'Academie des Sciences* now precedes everything it publishes with an extended abstract in English. ☐

RESEARCH SPENDING

Whatever happened in 1986?

CAN you see politics in a graph? Yes, if it is a graph of French spending on civil research (see right) The 'accordion' effect, as the French say, of policy changes in 1986 and, then, 1988 cries out for explanation.

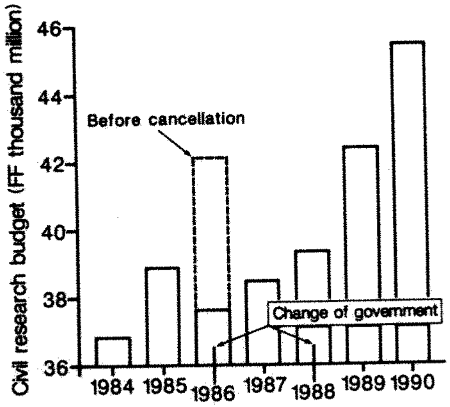
The present period is one of renewed growth after the cutbacks between 1986 and 1988, but the French research budget is only just finding a character of its own. State research spending, and how this is reflected at the laboratory bench, still bears the imprint of the exuberant policies of the young Socialist government elected in 1981 and then the sober pruning of a neo-Gaullist government that held office for two years from 1986.

But the tone of the decade as a whole is undeniably that set by the Socialist government formed by the newly elected president, François Mitterrand. In 1981, his new Minister for Research and Industry, Jean-Pierre Chevènement, organized a series of now historic colloquia (*assizes*) to help determine the shape of research policy, spawning radical initiatives that found their way onto the statute book in the 15 July 1982 law on the orientation and programming of science and technology research.

The government declared that civil research spending should rise to 2.5 per cent of GDP (gross domestic product) by 1985. To that end, the 1982 statute committed the government to increase the civil research budget by a remarkable average of 17.8 per cent a year and to increase the number of state-supported researchers by 4.5 per cent each year until 1985.

In 1983, Hubert Curien, the present Minister for Research and Technology (see next page) took over from Chevènement, and innovatory schemes were announced to encourage technological progress. A tax incentive scheme to promote industrial research was set up with the goal that, by 1985, industry should contribute 1.5 per cent of GDP to research and development.

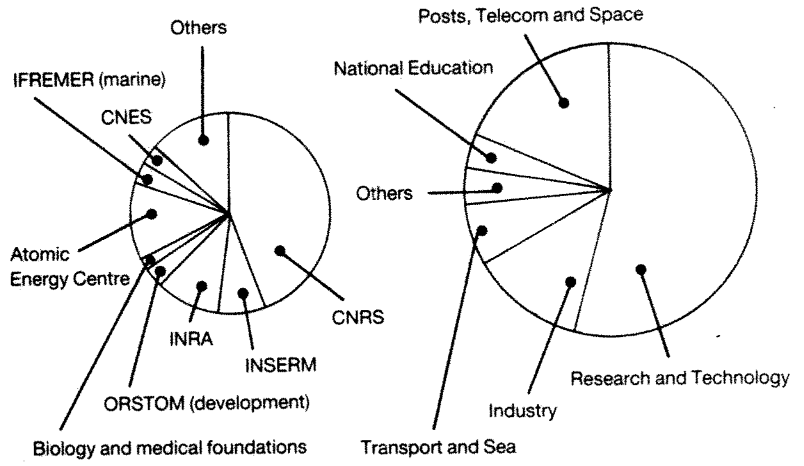
EUREKA, the now highly successful European Communities initiative to promote trans-frontier collaboration in high-technology industry, was proposed in 1985 by Mitterrand, but it was Curien's brainchild.



It would be facile to say that this early period was uniquely good and that it came to a sudden stop when, in March 1986, the Socialists lost power. (Mitterrand remained as president, during an uneasy spell of what was called cohabitation.) But the neo-Gaullist government led by Jacques Chirac sought above all to curb public spending and to encourage the private sector to carry more of the burden of research support. The 1986 budget was annulled and the budgets of the large research organizations, such as CNRS and INSERM, virtually frozen.

Whatever the economic merits of the Chirac government's drive to streamline what it regarded as a costly and overweight public sector, those were lean years for state-supported basic research. CNRS, long regarded as a hive of left-wing trade unionists which had become too large and too powerful, was at the centre of a maelstrom.

The ministries for research and for higher education were merged under Alain Devaquet who, with encouragement from conservatives inside and outside government, launched a plan to reform CNRS. But this failed, leaving the research council's affairs in turmoil for over a year.



The Research and Technology Ministry receives just over half of the government's civil research budget (right). The CNRS and its institutes dominates the ministry's budget (left).

Devaquet resigned and was replaced by Jacques Valade, but with a much-diminished ministry; most of the budget had been handed to the minister for industry. In the event, Valade achieved little; his appointment was followed by elections which, in 1988, brought the Socialists back to power, with Michel Rocard as Prime Minister and Hubert Curien once again in charge of research.

After a short period when higher education and research were lumped together, Curien was given his own ministry of research and technology from December 1988. The civil research budget, which groups together state finance from all ministries, was reinstated — and the Chirac government was accused of budgetary deception, using defence spending to mask a fall in civil funding.

With Mitterrand once again declaring research and education national priorities, Curien was able to inject FF830 million into the research budget immediately to offset the cuts of his predecessor. The 1989 budget was up by 7.6 per cent, recruitment began again and, in the 1990 budget, salaries were improved.

But the growth in the public sector is more like half that of the pre-1985 period. Furthermore, Curien has made it clear that he wants to encourage both basic and applied research, but that big organizations such as the CNRS must run a tighter ship. A committee for evaluation has been set up and plans to 'modernize' the CNRS are under way.

At the research bench, all is not rosy. With inflation running at 3 per cent a year, the 7.3 per cent increase of the 1990 budget amounts to a more modest 4.3 per cent in real terms. The figure is even less when costly space and aeronautics, which were given a special boost, are subtracted. And, because the number of researchers has continued to rise throughout the decade, but faster than budget increases, the average scientist's research money buys almost 20 per cent less than ten years ago.

Yet the present government has not wavered in its objectives. It wants to make sure that France is well placed for the year 2000. And it now aims to see national civil research spending — hovering around 2.3 per cent of GDP — rise to 3 per cent.

Peter Coles

SEARCH MINISTER

Curien weaves beguiling web

M. Hubert Curien is an unassuming, almost shy, man with the qualities of a terrier. He was summoned from his post as director of the Centre Nationale d'Etudes Spatiales (CNES) to superintend French civil science on the break-up of Chevènement's ministry of industry and research in 1983. During the Chirac interlude (1986–88), he was elected president of the European Science Foundation, but then summoned back to Paris as minister of research and technology when the Socialist party was returned to power.

Curien believes that French science has, indeed, become stronger over the past decade, partly at least because of his government's willingness to spend money on research. But he insists that research had not been neglected in earlier decades. One subtle change, he believes, is that the French are now proud of their research institutions. In the old days, "we were proud of M. Pasteur, not of the Institut Pasteur."

Links between basic research and industry have also been strengthened, partly at least because of the government's willingness to support (through, for example, CNRS joint projects on a 50:50 basis. Curien is pleased with the way in which CNRS has functioned as a focus for these activities. The interaction has also encouraged French companies to spend more on applied research. "If we had not done this, we should have achieved nothing", Curien says.

Unassuming terrier Hubert Curien.

Another striking feature of the past decade has been the steady internationalization of science — "it's now a real thing". He believes that French science has benefited powerfully from the impetus that has driven individuals into international collaborations.

He notes that French research groups profit greatly from the relatively small sums available from the European Commission, whose spending on research has grown to 4 per cent of total European spending on research.

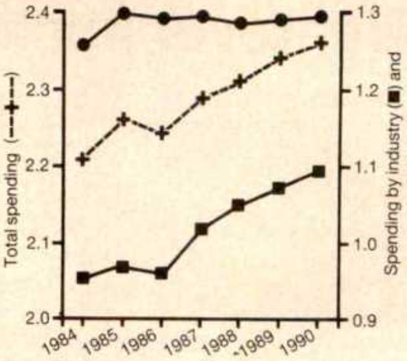
Curien also acknowledges that problems remain. One is mobility or, rather, the lack of it: people tend to stick in the same laboratory or other institution for too long. The concentration on Paris, around which something like a half of all French scientists work, is another.

But except for the salaries of beginning scientists at public laboratories, and the bottleneck in promotion in the middle ranks (which he is trying to widen), Curien is not too worried about the general unflattering comparison between salaries in the public laboratories and elsewhere. "If industry took more good people out of CNRS, I would be happy."

On the general opinion that France needs a substantial scheme of postdoctoral fellowships for its own nationals, he is ambivalent. He acknowledges that postdoctoral fellowships would encourage mobility within the network of government laboratories. Such positions would also help to improve teaching and research at the universities. But, like many others, he is impressed by the experience of 1969, when the then government agreed with the demands of trade unions that postdoctoral fellows of longstanding should be given permanent positions. "That", Curien says, "was a catastrophe."

Curien's position is powerful because his ministry is powerfully placed at the centre of the scheme of things. Other ministries (except defence) must say what they spend each year, and how. This enables him not merely to shape the general balance of research activity but also to back small-scale initiatives. A few months ago, for example, he was putting his weight behind a scheme due to Jean-Pierre Changeux, professor at the Institut Pasteur, for marrying French neurobiology with mathematical studies of the properties of neural networks; according to Changeux, he had mentioned the goal to Curien at a chance meeting. And about now, Curien will be giving a final opinion on an attempt to define French involvement in the Human Genome Project. (Many influential people are now less cool than previously.)

Others of Curien's schemes are less particular. Thus he began a programme (now costing more than FF1,000 million a year) enabling intending researchers, many of them industrial engineers, to write PhD theses by means of a three-cornered partnership with a public or university laboratory and a company. More recently, he has set up a national committee for the systematic evaluation of French science and technology, which in turn has spawned a small unit called l'Observatoire des Sciences et des Techniques, bent on the development of indices of performance.



Figures (% GDP) reveal that industry takes the credit for much of the recent increase in spending on research.

Yet one of the ministry's goals remains beyond Curien's reach. It has long been the Socialist government's view that research spending should amount to 3 per cent of GDP, putting France on a par with West Germany, the United States and Japan. This year, the percentage is 2.38 per cent (see figure). The ministry blames the shortfall on France's industrial enterprises, whose spending on research has grown, but not as quickly as the government asks. But the figure shows that it is the government's spending that has stagnated, as a percentage of GDP, at just under 1.30 per cent.

But Curien's ministry does more than merely administer science and technology; it is one of the chief means of persuading France's taxpayers (other than those who get tax credits for research spending) that science and technology are good for them.

Pride of France: the Pasteur has at last ousted Pasteur in the scientific hierarchy.

Thus a recent popular (and handsome) booklet on French science under the title "A new impetus (*élan*) for research" echoes the president of France, François Mitterrand, by declaring that its goal is to "make research a national priority". Research, it declares, "is at the heart of the development of modern societies. . .". Research will enable France to "meet the challenges with which the national economy will be confronted in the global market" and to "keep its place among the principal industrial powers of the planet".

Nobody at the research ministry is shy of this rhetoric, which everybody believes. And, of course, there are many in research who hold that the grand phrases are merely a statement of the obvious. But the French research ministry differs from comparable organizations elsewhere in taking such a a positive stand in favour of the ways in which it spends its budget funds. It is good for the morale of those who work in the ministry's dependent organizations, and may even have helped to cultivate the general French interest in all things new and technological. Curien, who is in no sense a salesman in the conventional mould, may have done more than he imagines to engender enthusiasm for French research. □

EMPLOYMENT

Working on the public payroll

One way or another, most French researchers work for the government. There are more than 22,000 of them (and 37,000 support staff) on the payrolls of the research councils and the other public organizations active in civil research. (The Commissariat à l'Energie Atomique or CEA, for example, employs more than 1,400 people on research as well as 4,000 on research support.) The numbers of qualified scientists and engineers at the universities may be even greater.

For what it is worth, employment in the public service is growing overall. This year, the research ministry estimates that 432 new posts in research have been created (offset by the loss of 31 posts at the CEA). This amounts to a growth of research posts of 1.9 per cent. The growth of support staff between 1989 and 1990 was roughly half as much.

Luckily, the members of this small army of people do not all behave as civil servants. The republican spirit, as well as the common name *chercheur*, gives them independence. Many are prepared to bite the hand that ultimately feeds them.

Otherwise, industrial research and development is a growing source of employment, as are international organizations as different as the Institut Laue Langevin at Grenoble (see page 137) and the Joint European Torus (the European Commission's thermonuclear fusion laboratory) at Culham in Britain. One also senses among French researchers a growing interest in working at public research laboratories elsewhere in Europe; the Max-Planck institutes are most often mentioned.

In France itself, there is also a handful of institutions which, while largely supported by government funds, enjoy an exceptional degree of autonomy acquired either by tradition or by access to an independent source of funds. One obvious example is the Collège de France, created in 1530 by the then monarch François I to enable him to keep up with the scholarship of the Renaissance, and which is now a means by which outstanding researchers can both redouble their efforts (by setting up extra research groups) and supplement the high-level teaching provided by the universities of Paris. Another is the Institut Pasteur, founded by Louis Pasteur with the profits of his vaccine business in the nineteenth century. (The Pasteur's smaller and less well-known cousin at Lille, with its special interest in tropical diseases, is a far from negligible institution.)

Salaries are more or less uniform throughout the research organizations, but may be supplemented (to a degree widely considered as "unimportant", often "derisory") by part-time university teaching. The present salary structure in CNRS strongly emphasizes the importance of the promotion barriers in the determination of researchers' well-being.

So the research ministry had promised a three-year promotion plan that will loosen the structure. Beginning in 1991, those who have spent four years on the lowest rung of the research ladder (in the grade *chargé de recherche de deuxième classe*, or CR1), will be promoted to CR1 against evidence of their competence in research. The budget for the current year also allows for a shift in the balance between the three senior and two junior research grades, the effect of which will be to replace 462 CR2 research posts by a similar number in the senior grades (DR1, DR2 and DRE, where DR means "*directeur de recherche*" and E stands for "*exceptionel*").

There are similar proposals for improving the promotion prospects of support staff, so that an extra FF120million is spent on salaries this year. Yet the employment of support staff is a widespread headache for laboratory directors. Even secretarial jobs must be filled competitively, by *concours*. Technician-engineers are usually able to do better for themselves in commercial industry than in the public service. Computer experts are almost impossible to recruit on the salary scales on offer, with the consequence that many researchers are reconciled to writing their own computer programs (while others skew their industrial collaborations so as to get help of this kind). □

ORGANIZATIONS

Playing the name game

Acronymism has become part of French culture, the influence of the Académie Française on the language notwithstanding. Prudent organizations publish lists showing what the acronymic names for their dependent units mean. What follows is a guide to the principal French research agencies and their dependent parts.

Organizations in France quickly acquire a life of their own. To create a new organization within the public service is no easy matter: the whole government has a say, and the Council of Ministers must approve. But once created, an organization cannot easily be dissolved. Both the bureaucracy and the unions will use their influence in the interests of permanence.

The Centre National de la Recherche Scientifique (CNRS), which celebrated its fiftieth anniversary last year, is the oldest and the largest. Its purpose is the conduct and encouragement of basic research in the whole of science. To that end, it employs 26,000 people, more than 11,000 of them researchers. Its public subvention in the current year is FF10,330 million, up 6.9 per cent from the previous year.

INSERM stands for Institut National de la Santé et de la Recherche Médicale and operates on a scale roughly one-sixth that of CNRS. The current budget (FF1,830 million, up 5.6 per cent from 1989) allows the employment of more than 1,900 researchers. Many of INSERM's research units are free-standing laboratories, more often (as at Lyon, for example) sited near hospital complexes than university student campuses. The interests of these two research councils deliberately overlap. CNRS takes the whole of research for its canvas, but there are several joint CNRS/INSERM research units.

INRA (for Institut National de la Recherche Agronomique) will cost the research ministry FF2,450 million this year (an increase of 5.0 per cent). Its operations are more self-contained than those of CNRS and INSERM, both of which are increasingly dependent for research funds on partnerships with other agencies and industrial organizations. Its 1,670 researchers are only one-sixth of its total manpower, reflecting INRA's role in providing agricultural extension services. □

CREDITS

Money oils the wheels

SINCE 1983, France has offered industrial and commercial enterprises an abatement of their tax bills on account of the research they carry out or otherwise support, but on a formula devised by Jean-Pierre Chevènement that must often seem a little like that of the mediaeval torture apparatus called the rack. The tax credit is calculated not on the basis of what enterprises spend in the relevant year, but on the extent to which their research spending has increased from one year to the next.

How well has the system worked? A study last year by two industrialists (Jean Cantacouzene, director of research at the oil-company Total, and Pascal Gendreau, joint director-general of the government organization for small and middle-sized business) has shown that the system at least provides a means by which the government can get to learn of the changing pattern of research in different kinds of industries.

One surprising finding is that tax credits tend to be a larger proportion of total research spending in traditional industries, such as furniture and textile manufacture and in food processing, than in high-technology industries, no doubt because the companies choosing to register for credits now have only recently embarked on such research.

The system is also cunningly biased against large enterprises. Of 346 companies with turnover exceeding FF500 million applying for tax credits in 1987, the credits eventually awarded amounted to only 2.5 per cent of total research spending, compared with 12.8 per cent for the 3,682 companies with a turnover of less than FF500 million. It also seems that the smaller businesses constitute the fastest growing of the tax authorities' applicants, suggesting that the scheme serves its purpose of spreading the research culture.

The figures brought to light by the tax-credit scheme are part of the basis for the government's assertion that French industry is still spending less on research and development than it should be. The report of the Cantacouzene-Gendreau study concludes that industrial research is some 0.5 per cent of GDP less than it should be if France is to keep up with West Germany and Japan in relative terms. That deficit amounts to FF25,000 million a year at present.

Overall, the numbers of companies benefiting from the tax credit system multiplied fivefold between 1983 and 1989, to an estimated 7,000 (the returns are not yet complete). The cost to the Treasury is estimated to have been FF2,600 million last year — but the cost of administering the system is said to have been "derisory".

For the future, it seems to be agreed that the scheme should be given more publicity, and that the government should agree to make it permanent. Already it has been conceded that companies making substantial research expenditures in a single year should be able to spread the funds over several years. ☐

INDUSTRIAL RESEARCH

Can government back winners?

FRANCE has taken a long hard look at Japan's success, and wishes it could follow suit. But, with French industry not willing to shoulder the responsibility for spending what it might, the government has become the initiator of last resort.

That seems to be the philosophy on which the French government is now working, and the explanation for the substantial support for industrial research over the past two years. Between them, the research and the industry ministries will this year spend about FF5,000 million on industrial research of a general character, quite apart from the best part of FF6,000 million spent on research at the CEA and other mission-orientated research organizations concerned with space, aeronautics and the like. The largest single increase in the research ministry's budget for the present year — 30 per cent — takes its general fund for industrial research to FF1,565 million.

There have always been hankerings in that direction. The legend of the French nuclear energy industry began that way, with a deliberate decision to found a new industry with research and development. Did not France also invent EUREKA (in 1983) as a way of persuading other European states to invest funds in high technology?

Research minister Hubert Curien leaves no room for doubt that the sponsorship of industrial research is his priority for 1990, as it has been for the past two years. The emphasis will be on the search for new products and processes, to which the ministry expects to commit FF300 million this year, on perhaps 40 projects. The chosen fields are in new materials and food technology and biotechnology.

Two other goals have high priority, of which the more ambitious is that France should make a mark in the development of high-definition television (HDTV). It is only a small part of the calculation that France itself is likely to be a natural market for devices that bring the clarity of the cinema-screen into every living room.

More relevant to the choice of this priority is France's conception of itself as Europe's research strategist. There have been several occasions in the past 30 years when France has invested its own funds in huge technical projects it could not hope to carry through on its own, in competition with other players on the international markets, but which have been strategically important for Europe.

Nuclear energy is such a case. Although there has been a brief pause in French reactor construction (see page 139), if — some would say when — there should be a revival of demand for nuclear generating capacity in Europe, French nuclear contractors may be better placed than others to win orders from European utilities.

Much the same has happened in aeronautics and space launching, where French investment in research has been used (together with political persuasion) to prompt the formation of international companies that manufacture civil aircraft and offer space launching facilities (Airbus Industrie and Arianespace respectively). In each case, the objective has been to create a European counterpoise to a *de facto* US monopoly.

French support for HDTV is, rather, directed eastwards. From the outset of the EUREKA scheme, several projects have been directed at the processing of digital representations of video images, the basis of schemes for radically improving video definition. Non-French companies such as Siemens (West Germany) and Philips (Netherlands) have been closely involved from the beginning. But nobody should be surprised if official French enthusiasm for HDTV — the EUREKA rules allow governments to contribute towards companies' research expenses — culminates in a proposal for a joint European venture to head off the Sony's of this world.

The processing of silicon wafers is the research ministry's other immediate goal. The code-name is JESSI (for Joint European Submicron Silicon). The immediate goal is to make a 1-Gbyte memory chip, but the hidden agenda might be to make a *super*computer on a single chip. Modest Hubert Curien's ministry will acquire clout (perhaps even kudos) if the enterprise succeeds because of its ability (relative to that of comparable European ministries) to urge its client companies towards investment by offering (sometimes threatening) to spend its own money.

For the rest, the ministry plans to concentrate on small and middle-sized companies, traditionally cared for by a state agency called ANVAR (for Agence National de Valorisation de la Recherche), whose budget has been increased by 10 per cent this year. It will be interesting to see, a year from now, how many of those intermediate companies are in the business of high technology. The guess of 100 per cent will not be far from the mark. ☐

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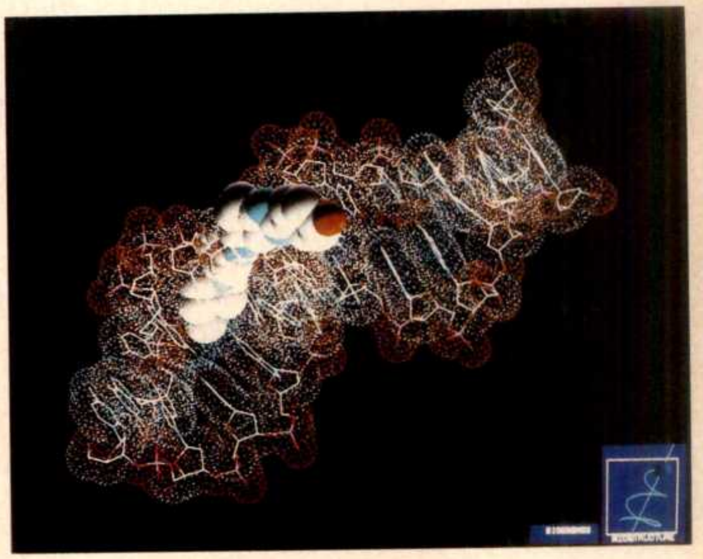

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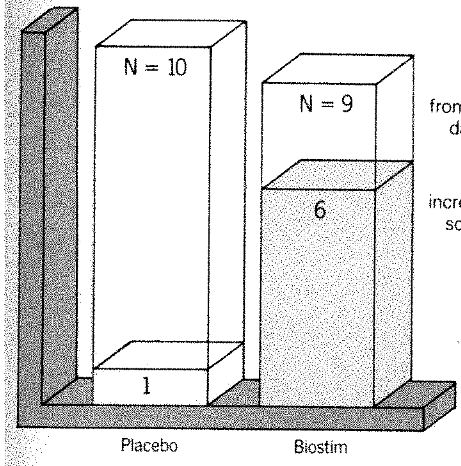

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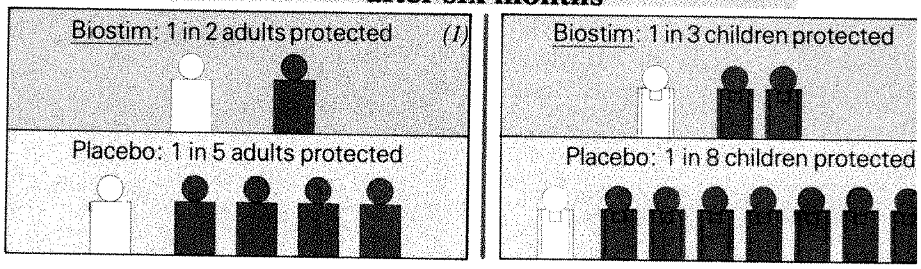
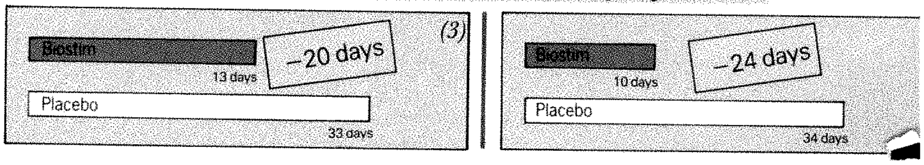
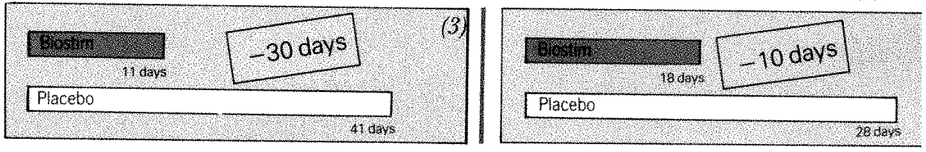


Reduction in average length of infections



Reduction in average length of treatment with antibiotics



(1) *Prof. Chrétien 52 cases — Ref. Rev. Pneumol. Clin., 1985, 41, 3: 213–217*
(2) *Prof. Pech 78 cases — Ref. Cahiers ORL, 1987, XXII, 3: 217–220*
(3) *Prof. Boutin 73 cases — Study over 9 months, Ref. Poumon Coeur, 1983, 39: 53–57.*
(4) *Prof. Paupe 42 cases — Study over 6 months Ref. Ann. Pédiatrie, 1986, 33 (9): 843–845*

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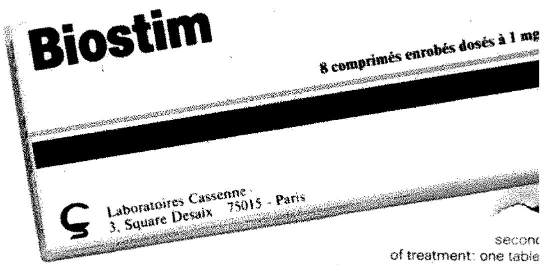
(Prof. Griscelli — Prof. Akoun — Prof. Paupe — Dossier of A.M.M.)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CNRS

To modernize and open up. . .

FRANÇOIS Kourilsky, the molecular biologist from Marseilles who is now director-general of CNRS, was appointed to his post by Hubert Curien in 1988 with firm instructions to "modernize and open up" CNRS. How well does he believe the task is going?

He is anxious that the organization should not be mistaken for the whole of French science, noting that it accounts for less than 19 per cent of the total civil budget. But he is pleased about the growing links with industry. At the latest count, industrial contracts with CNRS laboratories number 2,700, nearly two for each of the 1,300 distinct research units. Their total value is FF1,300 million, more than a tenth of the total budget. There is, he believes, a long way to go before the laboratories will be at a loss to know where next to sell their services.

Industrial contracts seem to serve a wider purpose than to keep the wolf from the door of some CNRS laboratories. To this outsider, one of the wider consequences has been to persuade industrial companies that there is, indeed, some benefit to be won from research and development.

Kourilsky says that, until recently, French industry has not significantly increased the numbers of people it employed on research and development. But now, he says, there is a surge in the recruitment of people, especially in fields such as mathematics, computer science, electronics and chemistry, not to mention molecular biology. Evidently he shares Curien's view that those who work for him should be doubly honoured if they leave to work in industry.

But is there a danger that the balance between contract work and basic research will be too much skewed against the latter? Not as things are, he holds. Perhaps the difficulties will arise when, as the volume of contract work continues to grow, but patchily, some laboratories will find themselves short of people for their core programmes while other, lacking contracts, will have people but only modest research funds.

Kourilsky agrees with Curien (see page 126) that the salaries of young researchers should be improved, but considers that the promotion bottlenecks are "still a problem".

On the scheme to provide university teachers with research funds through a committee within the education ministry, he holds that the source of funds should be independent of the university system. "On that, I diverge from Claude Allègre." The problem is that of evaluation. He regrets that the Napoleonic universities "lack independence".

CNRS differs from most comparable organizations in the large number (1,300) of laboratories in which its people are dispersed. The range is from a handful of people to some scores. So how are new laboratories started, and old laboratories disbanded?

As always, it is easier to start than to stop. The process of evaluation, Kourilsky believes, is accurate and reliable. But the law requires that a decision either to open or close a laboratory should be taken only on the basis of expert advice. The process can be slow.

On the future of the organization, he believes that links with universities will be strengthened, together with those with industry. But there are particular opportunities, he believes, in the emerging pattern of a single Europe for forming stronger links with overseas laboratories.

Kourilsky has not been at his job for long enough for his impact on CNRS to have become clear. Moving the bureaucracy will not be a simple task. But he seems to have one important augury on his side — a largely enthusiastic research force. □

BUDGET PRESSURE

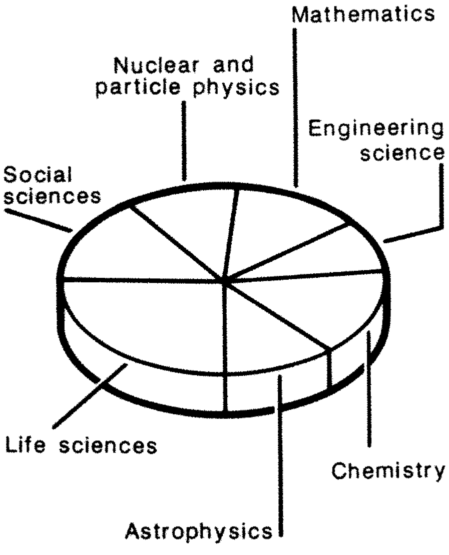
Staying ahead of the game

THE albatross around the neck of CNRS is the risk of being blamed for everything that goes wrong.

Although, on balance, CNRS attracts more praise than protest, the years ahead may be more difficult. The budget squeeze on CNRS's disposable expenditure is one source of strain. The need for some means of financing university research will be a further complication.

CNRS is potentially the more vulnerable because it has a finger in every pie. The existence of INSERM notwithstanding, for example, the life science division (one of seven) takes a quarter of the total budget. But CNRS is also strong in the humanities and social sciences. (The Science de l'Homme et de la Societé division takes more than 10 per cent — see figure below.)

Over the decades since the early 1960s, when it was common to find CNRS researchers working in isolation, and on a shoestring, in cubby-hole laboratories throughout the University of Paris, the organization has shown itself to be remarkably resilient and adaptable.



Distribution of CNRS funds between divisions, 1990.

The Chevènement upheaval of the early 1980s may nevertheless be one of the best things to have happened to CNRS, confirming its central place in the French scheme of things as well as the role to which it had already aspired of being one of the chief means of research planning in France.

The then-new director-general of CNRS, Pierre Papon, made forward planning his centrepiece. After an elaborate consultation within CNRS and industry, a score (literally 20) research themes were singled out for special attention, and became the basis for research planning in succeeding years. While the interest of the particular themes may since have been attenuated, the mechanism remains. CNRS is forever organizing consultations among interested groups to determine what weight should be given to particular themes.

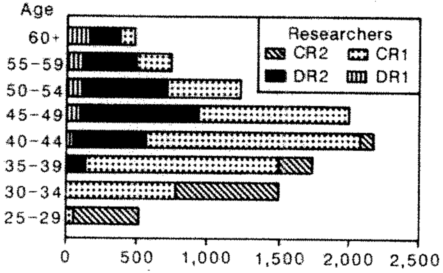
During the same period, the influence of CNRS on university research has been formalized and legitimized, by the device of associated (*associés*) laboratories, set up by means of formal contracts between universities and CNRS, and in which CNRS and university employees work side by side. (Other research organizations, such as INSERM and CEA, follow the same practice.) Between them, the research agencies have come to provide support — people as well as money — for most of the outstanding research at French universities. Throughout France, CNRS is this year supporting 1,003 research groups by this mechanism. (It also has 366 in-house research units, some of them very small.)

There is no shortage of academic research groups looking for support of this kind. By a curious device invented in 1982, the education ministry nominates (on the advice of CNRS) research groups considered deserving of outside support — *recommandé* is the designation — which may look for such crumbs as fall from the tables of CNRS and the other *grandes organismes* of research, perhaps end for full support. On one reckon- there are a further 1,000 of these earch groups waiting in the wings.

Now, as always, the snag is the budget. Despite the general growth of funds for research, the CNRS share of the civil budget has not grown as quickly as the whole. And the commitment of nearly 75 per cent of CNRS's share to the salaries of its employees means that its disposable income is shrinking. On one estimate, annual research expenses at CNRS account for FF110,000 a researcher, compared with FF180,000 at INRA and FF215,000 at INSERM (the best heeled of the research-support organizations).

CNRS seems bent on righting that imbalance. University research groups, especially the *recommandés*, were chilled at the end of last year when Kourilsky (see previous page) remarked that "CNRS does not have the means to support all university research groups" even when they are excellent. That sharpens the dispute over the education ministry's plan to provide research funds direct, but it also requires that CNRS itself should choose between being the daring cat that steals the cream from the top of the milk and being the pillar of the establishment it has become used to being.

That is for the years ahead. Meanwhile, CNRS has kept its options open. The organization is typified by its post-Second-World-War headquarters at Gif-sur-Yvette, in the suburbs southwest of Paris. The 'campus' is a park, acquired cheaply in a deal that requires CNRS to keep a team of gardeners on its books so as beautify the park. On one side is a fairly recent chateau (now a visitors' hostel) and, on the other, a collection of laboratories embodying the recent history of CNRS in bricks and concrete (mostly concrete from a less prosperous age). Despite the gardeners, the campus seems seedy.



Age-structure of CNRS research population, 1988.

What CNRS has been doing is plain enough: it has been following trends apparent elsewhere. Although nuclear and particle physics are still responsible for FF790 million, the proportion has fallen from 22 per cent in 1978 to 10.9 per cent in 1989. But life sciences, less than 18 per cent in 1978, are now 25.3 per cent (and employ more than 2,800 researchers at CNRS in-house and out-house units). Inevitably, the life sciences division of CNRS spends more of its budget on recurrent costs than on equipment and (less predictably) just about half of its total budget on associated and joint (with other councils) laboratories. In the first half of the 1980s, engineering sciences (especially computers) grew quickly — and the number of research posts virtually doubled — but now chemistry is the most quickly growing.

CNRS has also been ageing. In 1988, only 35 per cent of its researchers were younger than 40. Although the recruitment and promotion rates are now to be increased, it is difficult to see them quickly creating the bottom-heavy pyramidal age-structure one would expect to find in a research organization. No wonder that Curien (see page 126) says that he is glad that industry threatens to hire away senior people. ☐

RESEARCH CULTURE

A club-like atmosphere

THOSE who work for CNRS behave like members of a club because they are members of a club. To be a CNRS researcher is to be a member of a distinctive group, as if it were to be the French Navy or the *corps diplomatique*. But CNRS is, of course, not quite as grand.

The sense of club is easily explained: for at least the past decade, entrants to CNRS have won their places in the public service by competition in the common *concours*. They vividly appreciate the distinction between themselves (who are in) and those who are out. At least until recently, they could be reasonably sure that they would work permanently in this branch of the public service, with occasional postings elsewhere.

But this commonality does not engender a dull sense of uniformity. Different research laboratories have their own distinctive spirit. Some are plainly riding high, perhaps sustained by industrial contracts or grants for special projects. Others harbour a deep sense of neglect. They are inevitably the laboratories most oppressed by the bureaucracy of their parent, which is organized into seven divisions and no fewer than 36 sections, each with its own secretariat.

The underlying difficulty is the small proportion of the CNRS budget free for research expenses. More than 75 per cent of the budget is committed in advance to salaries; heat, light and maintenance take a substantial proportion of the rest. (INSERM seems better placed in this regard.)

Travel funds are not too hard to come by; most people acknowledge they can make one substantial overseas journey every year or two, while European Communities sources are increasingly used for travel within Europe. But the proportion of a laboratory's total budget remaining in a director's hands for research expenses may be relatively very small, perhaps less than 10 per cent of his salary bill.

Yet CNRS abounds with people who hold the highest opinions of their organization. For many, the sense of security and freedom they enjoy makes up for the gap between government and industrial salaries. One, the director of a laboratory at Gif-sur-Yvette, says that CNRS provides unique opportunities for research. Another gives it as his opinion that CNRS, whatever its faults, has become the dominant arbiter of quality in French science. The organization's willingness to enter into collaborations with universities and industrial companies, sometimes taking the initiative in forming them, is generally commended.

One British member of a joint CNRS/INSERM laboratory says that he is more settled, and more productive, in France than he had been at a British university. He reckons that the continuing shortage of academic positions in Britain must soon stimulate a soutwards stampede across the English Channel when British people realize that, at the outset, all the French they need is that required to keep body and soul together.

Another Briton at another laboratory, fresh from a postdoctoral position in the United States and with an enviable reputation for the development of a new technique, says that he chose the CNRS laboratory from among half a dozen at which he had been offered a position. (Sybaritic considerations seem to have played a part in the decision.) Testimonials of this kind abound. Because, to paraphrase *Anna Karenina*, contented employees are all alike, but discontented employees differ in their discontent. The following complaints against the organization are necessarily more voluminous. Their balance should not be judged by the space they occupy.

First, there are problems about people. Laboratories not well-endowed with outside funds are less able than others to keep competent young people at work while they compete for permanent places at successive *concours* for permanent positions. The result, some say, is that able people are often lost to the system at the beginning of their careers.

Filling vacancies is a constant headache. If a vacancy should arise, even among the technical or administrative staff, it cannot automatically be filled. Instead, the section or even division to which the laboratory belongs will at least consider whether the vacancy should instead be awarded to some other unit. According to at least one director, there are no mechanisms for appointing even junior staff on a temporary basis; either a public servant, or nothing.

There are recurring problems about research allocations. Laboratory chiefs are usually told, in the February of the year to which the information applies, what their allocation of funds will be. One CNRS director says that last year, after his budget had been cut by 30 per cent with no explanation, it took him until November to get the cut restored. (Luckily, the rules do not require that funds should be fully spent in the year for which they are intended.)

The need to make visits to Paris regularly, either as a member of an advisory committee (which is essential for staying in the swim) or to lobby against some particular injustice, is particularly irksome for those who live and work elsewhere. Reimbursement for the cost of doing so, as for other expenses legitimately incurred on CNRS business, is generally agreed to take months. One researcher says that he expects that CNRS will at any stage owe him FF20,000, or a month's salary.

Although many CNRS laboratories on university campuses enjoy the respect of and a good deal of influence with the associated universities, others lead a precarious existence there. One such group has been required to move its laboratory several times in the past seven years to suit the convenience of the university, and is now threatened with another move in October.

The most serious of the complaints against CNRS is that at least some laboratories appear to be inadequately informed of the overall policies that determine their affairs. This seems a shame when the people concerned are every bit as full of enthusiasm for their work as the general run of people working in CNRS laboratories. ☐

PLANT SCIENCE

Hungarian sowing seeds

THE Institut des Sciences Vegétales at Gif-sur-Yvette differs from others in the park in that it is brand new and that its director, Adam Kondorosi, is newly arrived, with his wife and co-worker, from Hungary.

The refurbished laboratory is that previously occupied by the teams who danced attendance on Gif's giant phytotrons, which were in the 1960s proof of CNRS's promise to botanists that they are deserving of large equipment as are nuclear physicists. The phytotrons have now been dismatled and the laboratory disbanded.

Kondorosi has been commuting between Gif and Szeged in Hungary for the past 18 months, while recruiting people for the laboratory. His appearence, more or less permanently, in this Paris suburb justifies earlier fears in Hungary that liberalization would entail the loss of able people (see *Nature* **344**, 611; 12 April 1990).

Kondorosi says that he is enjoying himself enormously, but that whether he will stay for good depends as much on his wife's inclinations as his own. Meanwhile, he says that he has continuing responsibilities at Szeged, mostly for the continuation of his research with graduate students, so that he will have to make occasional visits. CNRS seems compliant.

For the rest, Kondorosi's tale is an illustration of how CNRS (in this case with some help from INRA) sets about founding a new institute. The impetus was the conviction that the molecular and cell biology of plants would have important applications in agriculture. An international committee spent some months head-hunting for a director until it found Kondorosi (he rattles off the names of those who turned down the job). Now the objective is to recruit a team of people, perhaps internationally, to staff the five research groups whose work will stamp the laboratory's programme (and whose heads are already in place). The hope is to build up to between 60 and 80 people in four years.

One distinctive feature of the programme is that external collaboration is to be built in from the outset. Kondorosi says there are already agreements with the Max-Planck Institute at Köln and the department of molecular biology at Rome, not to mention his old institute in Hungary. He is also planning to work closely with the complementary CNRS institute at Toulouse, and is hoping for further support from the European Communities and from the European Molecular Biology Organization.

Kondorosi's own field is the study of how the symbiosis of rhizobia and plant roots is reflected in and mediated by the reciprocal exchange of chemical signals between them, which is one leg of the institute's programme. Another group will aim at similar understanding by more classical studies of the hairy-root syndrome (a variant of crown gall disease). There are also groups concerned with plant cell signalling (by means of auxin), the mechanism of damage by plant viruses and the molecular genetics of *Arabidopsis* — the eukaryotic plant with the smallest genome so far recognized.

Like other up-and-coming CNRS laboratories, the new plant sciences institute is already embarking on graduate education. Five or six students seeking the DEA diploma (a pre-PhD qualification — see page 122) have already been accepted for next year. Kondorosi expects that university relations will be simplified because two of his group leaders are also university professors.

But will there be enough teachers for them? Kondorosi says he has already advertised 18 vacant positions at the institute. There can hardly be a quicker way for someone from Eastern Europe to learn the ways of the competitive West than he has chosen. ☐

DEVOLUTION

Regional consuls in the wings

MME Katherine Piquet-Gauthier is the new regional delegate of CNRS at Montpellier, with responsibility for CNRS affairs in Languedoc-Rousillon in the south-west of France. She is one of a dozen people appointed last May, under the terms of a decree promulgated by CNRS last December, to offer regional groupings of laboratories with a more immediate presence than Paris can provide. But it is not yet clear how far administrative devolution will go.

The twelve regions and the sites of CNRS's regional offices are Alsace (Strasbourg), Aquitaine and Poitou-Charentes (Bordoaux), Brittany and Loire (Rennes), Limousin (Orleans), Ile de France (see below), Langeudoc-Roussillon (Montpellier), Lorraine-Ardennes (Nancy), Midi-Pyranees (Toulouse), Normandy (Caen), Provence-Côte d'Azur (Marseille) and Rhône-Alpes (Lyon). The administrative centre for the Pas de Calais region had not last month been chosen.

Many of the regional directors have not yet taken up their posts. The arrangements for the Ile de France (greater Paris) are complicated by the great concentration of CNRS responsibilities in the region, with the result that there will be five sub-regional directors working through a central office. Similarly, there will be two sub-offices in the Rhône-Alpes region catering separately for Lyon and Grenoble.

What will the regional offices and their directors be able to accomplish? Laboratory directors and researchers have a variety of aspirations for the new arrangements. Those who labour under a sense of neglect hope the new arrangements will give them a stronger voice in Paris. More immediately, there is also the prospect that the regional offices will simplify the bureaucratic structure (although some fear that their influence may be in the other direction).

On the question of whether devolution will make it easier to pay people's legitimate travelling expenses more quickly, Mme Piquet-Gauthier says "not yet, but wait and see whyat it's like when you're here next". ☐

ALUATION

RS reflects with pleasure

RS, which is always evaluating other ople, has recently been evaluating self. But the result is not so much a critical appraisal of the largest single research programme in France, as a kind of guidebook to science in general. The report of the evaluation is dominated by the theme of the growth of interdisciplinary research requiring multidisciplinary teams.

The self-evaluation, the report of which has just been published as *Rapport de Conjoncture*, has been prepared by 22 commissions established to evaluate themes singled out by CNRS for study, and ranging from Earth and Solar System to order and chaos and the transformation of societies. Readers must be prepared to look between the lines for outright self-criticism.

But one general theme is the emergence of a number of interdisciplinary problems to which French science should pay attention. One singled out for discussion is that of global change, but the study also notes that the properties of neurons promise to be of great importance in computer sciences as well as in physics and mathematics.

This colours the report's opinion of present arrangements for the training and recruitment of young people into science. It argues that there is a need to improve and increase the training of doctoral students to ensure a sufficient supply of researchers and university teachers. But doctoral training should also prepare young researchers for working alongside people of other disciplines. The report asks that something should be done to improve the public image of certain disciplines, citing mathematics (surprisingly) and chemistry as being unpopular.

On interdisciplinary research, most conspicuously at present represented in CNRS by four named research programmes (in materials science, energy, environment and technlogy at the workplace), the evaluation draws attention to some of the problems faced by those engaged on them, in particular by the risk that work on interdisciplinary problems may complicate the two-year evaluation of their research achievements by one or other of the 49 subcommissions, are responsible for this work. The report argues that people's willingness to work on interdisciplinary projects should be regarded, *a priori*, as favourable for their careers.

Of the international connections of French research, the evaluation acknowledges that English has now become the language of communication in international science, but says that there remains room for French on the national scene and in relations with francophone countries.

In general, the evaluation says, it has taken an optimistic view of the place of French science on the international stage, but it acknowledges that there may be a bias in the process of evaluation — research that is inherently good will be well spoken of, while that which is bad will leave little impression on outside critics.

The document acknowledges frankly that international collaboration entails the risk that researchers will be lost to France by means of a "brain drain" which is not confined to France. But it says that French collaboration in large international projects has an effect "at once dynamic and stabilizing". It says that the involvement of French researchers in overseas laboratories, and visits of overseas researchers to France, but particularly the exchange of scientists within Europe, "must be one of the keys to our successful development".

SUPPLEMENTARY INCOME

Living better by one's wits

The Napoleonic centre, it appears, can be flexible enough when there is cash to be saved. That seems to be why every laboratory in the public service seems to have a freebooter's licence to strike a deal on research with some third party, within the limits of a framework agreement. So laboratory directors at CNRS and others of *grandes organismes* have become entrepreneurs of a kind.

The results have often been spectacular. In some CNRS divisions, external support may be as much as a third of the total. Other ministries and public agencies provide more than half of this extra money, for projects as different as the provision of special research equipment and for research projects with a bearing on some development by a still nationalized industry — the High-Speed Train, for example. But particle and nuclear physics and the life sciences are almost exclusively financed out of CNRS funds.

It is also striking that France derives a substantial research support from international sources — in 1987, this amounted to 17.9 per cent of CNRS's disposable income, exactly the value of research contracts with industry. The European Community is a particularly important source of funds, both because participation in the major European research programmes carries public researchers into joint projects with industrial researchers and because the much smaller sums available for occasional travel from Community sources valuably supplement what CNRS can spend. ☐

Some rights and wrongs

Among the points made in CNRS's evaluation of itself are the following:

French groups have played an important part in the development of **particle detectors** at CERN and DESY since the success with Gargamelle in 1973, but steps should be taken to build on the experience of constructing the LEP injector at Orsay. Building a **4 GeV** electron accelerator, "no doubt in a European context", would allow important work with high-energy photons in **nuclear physics**. New instruments, in Hawaii and Chile will be followed by the Very Large Telescope (four linked 8-metre telescopes) being built by the European Southern Observatory, but French **astronomy** should be primarily concerned with the supply of theoreticians.

More generally, the subcommission on these topics expressed concern at the difficulty of preparing graduate students both theoretically and with a capacity to tackle unknown problems, with the "linear" careers of young researchers and with the continuing shortage of research funds.

The mathematics evaluation says the French **mathematics** school is "probably one of the best in the world" (which, by general consent, is fair). There are up to 2,500 mathematicians at universities, with only a tenth as many at CNRS. Now, the evaluation says, CNRS should be employing more mathematicians who are, it is said, less and less easily classified as "pure" and "applied". By way of proof, the evaluation draws attention to the invention, by engineer Jean Morlet, of the concept of wavelets (*ondelettes*, or convolutions of periodic and gaussian function as sets of elementary functions in which seismic signals can be expressed.)

Surface research is crucial in, for example, the electronics industry, and "France is now well-placed" by the provision of national facilities by collaboration between government agencies and industry. But the advantage could be in hazard because of the shortage of CNRS funds for medium sized equipment, while the immobility of people makes the development of effective research groups difficult.

In *informatique*, France is said to be strongest on the theoretical side and in the development of languages, while Japanese and US industrial laboratories dominate such fields as the development of an **optical computer**. Yet in both respects, the evaluation says that European collaborative programmes have a crucial role. CNRS is said not to have increased its numbers of specialists in these fields sufficiently. ☐

UNIVERSITY REFORM

A search for *égalité* in diversity

Claude Allègre, the bluntly spoken geophysicist who was director of the Institut du Physique du Globe at Paris VI until two years ago, could make his name with the quiet revolution in higher education on which he is now engaged. Or he could lose his academic friends.

Allègre, energetic and at once ambitious and impatient, has become director of higher education at the Ministère de l'Education Nationale (and de la Jeunesse at des Sports). But he keeps his hand in at the institute. He teaches a course and spends time in the laboratory. He hopes to manage every weekday morning next year. But even now he is working like a dog.

The task is herculean. By any standards, the university programme is calculated to astonish. In the academic year just ending, there have been 1.13 million students (or their equivalents as part-timers) at France's 76 universities, which means that 40 per cent of school-leavers at least begin on higher education. Allègre says that the ratio should be 60 per cent. "Then we shall be like the United States and Japan."

There is another driving force. The university system is what it is because of the student rebellion in the streets of Paris in the spring of 1968. Nobody forgets that the Left Bank was occupied by students for days on end. (Many of those same students are now, no doubt, civil servants.) Thus has been accepted the principle of open access: qualify in *le bac*, the popular name for the *baccalauréat*, the nationally standardized school-leaving examination, and there will be a place for you at a university. Naturally, the numbers grow.

Down to Earth: Allègre is keeping one foot in the university world.

So, inevitably, does the budget. In real terms (francs of constant value), spending on higher education has increased by 35 per cent since 1980, but half that increase has been squeezed into the past two years. The French government will have spent FF27,500 million (in present money) on higher education in the current year. Next year (the budget is just now being devised) it will no doubt spend more.

Allègre is not one to be dismayed by the immensity of his responsibility. He spells out the agenda. Build more universities (eight are planned, four in the greater Paris region called the Ile de France, four at places yet to be determined). Lift the spirits and the public reputation of academics by increasing the pay of able people, perhaps by 25 per cent. Recruit more graduate students, perhaps by offering them better stipends. Improve the quality of academic research by a system of competitive research grants administered by the ministry. Sign contracts with universities so that they can spend their budgets to the best advantage.

There are even plans to change *le bac*. Allègre is proud of a scheme to introduce students to chemistry by asking them to build molecular models; by the time they come to grapple with the laws of multiple proportions and the like, they will at least know what is meant by valency. There are also plans to introduce continuous assessment of students' performance in the awarding of *le bac*, if not in mathematics, then in other fields.

All this is meant to happen quietly. "We are making a revolution", but we say that "nothing much is happening". The more natural course would have been a "big new law", but that tendency is the "French disease", Allègre says.

Allègre does not hide from difficulties, but meets them head-on. Will not a system in which universities' activities are predetermined by a contract with the ministry mean that university freedom is curtailed even more than at present? On the contrary, says Allègre. Universities knowing what they want to do can negotiate an appropriate contract with the ministry, and then be more free than ever to pursue their goals.

He is passionate on this point, complaining that his republican compatriots have consistently failed to appreciate that diversity means *égalité*, and is not antithetical to it. The tendency has been to suppose that *égalité* requires that everybody should follow the same curriculum at the same kind of school. Emphasizing that the participation rate should be high (60 per cent) he says, "Look here, France is a Catholic country, and Jacobin and Napoleonic. . . in the end, the Catholic influence has won through."

Those are the forces in favour of uniformity and centralism. He acknowledges that universities as they are are too much alike. But freely negotiated contracts with the ministry could give universities the right and the opportunity to be different. The first negotiations with universities, in the west and south of France, are now under way within the framework of an elaborate set of parameters conjured by the ministry — 12 m^2 of space for a science·

ACADEMIC LIFE

A new vision or an old mirage?

Academics in France differ in their estimates of whether the government's new promises of support will materialize, but only because there are different degrees of scepticism. Most say, "We have heard all this before. We'll wait and see what happens." Many others will not wait.

What follows is honest reporting of academics' views adapted so as to conceal its sources, for reasons that should be obvious. (It matters less that academics are literally civil servants than that their senior colleagues can still powerfully influence their academic prospects.)

One group of 40 university teachers in literature — a group now responsible for the first-year education of 700 students — is asked (by the university administration) to take responsibility for 1,200 students. That would mean increasing the student to staff ratio from 20:1 to 30:1. There would be no extra teachers.

A teaching group of similar size, most of whose members applied for special contracts on the grounds of special expertise in teaching or research, has found that only two special contracts have so far been awarded. The recipients are both in their fifties, and are people of acknowledged distinction. One is so eccentric that he no longer teaches, and does not publish either.

There is a general complaint at the teaching loads of university professors; ten hours a week of lectures is commonplace. "That's impossible", says one professor at Paris VI. Such a load means that even the teaching must be skimped, while there is literally no time for research — and the funds for supporting research are steadily declining.

The position may be "even worse in Britain, but not in West Germany — there they seem to be spending money more intelligently". In France, there is a crisis. There are "not enough teachers, and those there are thinking of leaving", mostly for international organizations and industrial laboratories.

Other academics protest that the criteria for deciding promotion within the system are far from objective. A proposal that people's competence as researchers should be judged objectively, perhaps by publications records, was rejected by the ministry a year ago. ☐

, for example, but 3.5 m^2 for one in individual universities are making al claims in respect of extra things do.

The contracts, when negotiated, will run or four years which, a little to Allègre's delight, breaks the rule that ministries must not anticipate spending not yet voted by the National Assembly. (One guesses that the ministry's lawyers will deal easily with that subtlety.)

Allègre also agrees that French universities have been too conservative. Scornfully, he notes that molecular biology was taught at and by the Institut Pasteur long before it found its way into the curricula of the universities in Paris and that plate tectonics (his own field) remained in limbo for too long.

The ministry's mechanism for supporting university research is more controversial. Academics from all over France will compete for a share of a pot of money administered by a ministry committee, half of whose members are drawn from European countries other than France. Allègre is fighting for a 15 per cent increase of the funds available in the budget for 1991, now being drafted.

Might not a ministry committee assessing the quality of research proposals be tempted to use the funds at its disposal for solving other than research problems? Would not an independent grant-making agency be safer? Allègre does not agree. For one thing, the committee is international, but its subcommittees will also rely on CNRS assessments when making their decisions.

As proof that the system is working, Allègre notes that it has already drawn blood. "A lot of people are very upset — mediocre people and some universities." But he does not like the US system of competition for research grants; "indirect costs" are "ridiculous", as is the difficulty of finding support for long-term projects.

He is confident that "zero — nought" will be spent on "big programmes". But he would like to encourage research in innovative fields. He rattles off a string of topics — *informatique* in clinical medicine, molecular identification by physical means, cogniscience (the new name for neurobiology), the geophysics of the topmost 100 metres of the Earth's crust and the meteorology of cities.

So why, with these grand plans, does Allègre risk losing friends? Few will dispute the good sense of his ambitions. One worry is that the interventionist habits of the ministry will persist. More important (see below), there is a general fear that the funds for supporting Allègre's quiet revolution will be, as so often in the past, inadequate for the need and, perhaps, even hijacked by the pressing needs for general support in the university system. □

UNIVERSITY REFORM

On the way to open access

In the academic year just ending, there were 1.13 million students at French universities, an increase of 77,000 over last year. Next year, there will be more — just how many more will be known only when high-school students have been told the results of *le bac* this year.

For several years, the system has been growing at about 8 per cent a year. The mounting pressure of numbers is felt even in the highest reaches of the government — the official communiqué from the Council of Ministers (the French government's cabinet) announced that the building of 200,000 m^2 of extra space at universities had been sanctioned against the new academic year.

Since 1960, when there were a mere 215,000 students, the scale of French higher education has multiplied more than five times. The growth has been most rapid in the humanities, whose students account for more than a third of the total. Law and the *sciences économiques* (which include accountancy and management) account for more than 250,000 students. But in 1989 there were 192,000 students following courses in science and engineering, an increase of nearly a half since 1980.

As Mr Kingsley Amis would have predicted had he been French, more has meant worse. Faculties have not grown as quickly as student populations, so that ratios of teachers to students have generally declined. Harassed teachers at institutions other that the *grandes écoles* have been forced to skimp on research and scholarship as teaching and administrative burdens have increased. Who can wonder that many researchers in dedicated research laboratories scorn their nearest-neighbour academic colleagues? Or that, mostly for lack of suitable candidates, there were 2,000 vacant teaching posts in June 1988?

The pressure of numbers is greatest in the early years. Undergraduates follow a three-stage curriculum (the *premier*, *deuxième* and *troisième cycles*). The attrition rate at the end of the first cycle (for many, the end of the first year of studies) is high. For those who stay the course, the *troisième cycle* or fifth year is either a vocational course (such as the preparation for a career in teaching) or, in science fields, a preparation for research.

The education ministry is not for nothing the Ministry of National Education. It operates schemes for the national validation of degrees, the *licence* and *maître* qualifications of the first and second cycles in particular. Those with academic bent follow a course leading to a DEA (see page 122) qualification, previously a sufficient qualification for higher education work, possibly following that by embarking on a PhD thesis, lasting for two or three years.

The government's plan to put right th state of affairs rests on these components: **Salaries and careers.** Teaching posts will ordinarily be filled only by people with PhD degrees. Teaching salaries will be tied to those in the public service and a system has been instituted by means of which the salaries of academics with special responsibility and competence in research, teaching or administration may be augmented if those concerned are offered special contracts of employment by the education ministry. The ministry says it has received 15,000 applications for such contracts, the effect of which will be to increase the salary of a beginning academic from FF8,000 a month to FF13,000. Allègre (see page 133) reckon that the average uplift of salaries will be 25 per cent.

As part of the process of deliberately preparing people for careers as academics, the ministry has set up thirteen *centres d'initiation à l'enseignement supérieur* at which, it is expected, graduate students seeking careers at universities will attend for one week twice a year during the average three-year duration of a PhD course. Graduate students selected for these programmes will be paid extra (a total of FF9,200 a month), but will be expected to teach classes (usually in the *premier cycle*) and to hold small-group seminars while preparing their theses. The plan is to increase the 3,000 people at present following this programme to a total of 6,000 (implying an annual production of 2,000 academics in all fields); graduates of the *grandes écoles* will be extra. — **New universities**. Existing universities earn intangible credit at the Ministère National de l'Éducation by their willingness to fall in with the notion of expansion, but there are limits to what can be accomplished on existing sites. Montpellier (see page 138), with 50,000 students, is pondering whether it could take an extra 20,000; one solution would be to spin off first-year teaching to Nîmes, 60 km away. Nîmes, with ambitions to be a university-city in its own right, is not so sure.

So France, almost alone among industrialized countries, is embarked on creating eight new universities — four in Paris and four elsewhere. The new Parisian universities, which will be multidisciplinary, will be surburban universities — at Cergy-Pointoise, Versailles and Mame la Vallée et Evry with a dependent campus at Melun-Senart. But it is also planned to increase by rebuilding the capacity of the Sorbonne and other universities in central Paris.

The hope is that the four new provincial universities, the locations of which have not yet been chosen, are meant partly to meet the demand for higher education which is, even at present, unsatisfied in west and central France. (There is at present a net movement of students towards Paris, the southwest and the Lyon region.) The problem will be to persuade able academics to move away from the centre.

Research. The government acknowledges the need to "arrest the slow erosion of university research over the past several years". The ministry of education hopes to accomplish this by several means.

There will be a system of competitive grants for research and scholarship administered by a committee (the Conseil Scientifique de l'Education Nationale) under the Nobel prizewinning chemist Jean-Marie Lehn. Salary supplements for academic-researchers should work towards the same end.

It may also help that the government plans to encourage the emergence of new university centres of excellence. It has nominated a handful of places which, "by the quality of their research, the diversity of their teaching and the the attractiveness of their locations", may rival "Oxford, Heidelberg and Berkeley" in the united Europe of 1993. The ministry has so far nominated Grenoble, Strasbourg, Orsay-Polytechnique (southwest of Paris) and Toulouse; it promises further names — but a few of them — before 1993.

Organization. The ministry of education, at the Napoleonic hub of France, is used to redefining and rebalancing the interests of the centre and periphery. The new calculation is that four-year renegotiable contracts with the ministry of education will give universities an incentive to skimp on spending in fields in which costs are elastic, and to invest the funds they save where there are intellectual opportunities, or students to be recruited. The council of ministers, on the recommendation of the ministry, will continue to appoint the rectors of the universities.

Several innovations are promised. A study is under way (with the ministry of economics and finance) to see whether budgetary procedures can be radically simplified and whether real-time ("*en temps réel*") techniques of data processing can assist the administration of universities. (Ways of counting students would be a big help.)

Cosmetics. The ministry, which hopes that it will be possible to double the number of doctoral students preparing theses in the five years ahead, plans to set up an organization for monitoring all theses under preparation. It is hoped that this information (to be published annually) will form the basis for determining university research policy as well as for comparisons between French and other research. ☐

Lyon's hot-house university

WHY should French teenagers compete so fiercely to go to universities that are unable, by their constitution, to award degrees? Because the universities are not universities at all, but *grandes écoles*.

These citadels of French higher education owe their existence to functional considerations: how best can the state secure the services of able people who have been specially trained in certain fields? By recruiting them young and competitively, by making them civil servants for at least ten years and then, by training them. Thus was the *École Polytechnique* Napoleon's device for assuring a supply of artillery officers.

By definition, the *grandes écoles* are in Paris, or at least they were. But in the 1980s the ministry of education embarked on an experiment to see whether they could be transplanted to provincial soil. Now, after more than a decade of heart-searching and hard work, a version of the *Ecole Normale Supérieure* has been transplanted from Saint Cloud, in Paris, to a complex of post-Modern buildings on the site of an old abattoir in this provincial city, now the next largest after Marseille.

The Ecole Normale Supérieure de Lyon has taken in 100 students in each of the past two years. They are a cut from those successful in the national *concours*. Most give themselves an extra two years preparation after *le bac* before entering the competition, which means that *normaliens* are usually 20 years old when they begin their studies. Although most of those entering the annual competition give one of the Parisian schools as their first choice, they (and their teachers) know they come from the top 0.1 per cent of whatever proxy for the national IQ distribution is measured by the *concours*.

Moving the school from Saint Cloud to Lyon has not been trouble-free. The idea seems first to have been mooted in the mid-1970s, and more or less agreed by the arrival of the Socialist government in 1981. At that stage, those threatened with banishment from Paris, and their unions, pleaded with the new government to put a stop to the plan. But the then minister of education, having brooded for some time, decided not merely that the move should go ahead, but that Lyon's floor-area should be increased by 11,000 m^2 to a total of 36,000 m^2.

Guy Aubert, appointed director of the new school two years ago, is an energetic man; among other things, he reckons to travel 50,000 km a year on the autoroute between his laboratory at Grenoble and the school at Lyon. He has high ambitions, not least that of making Lyon the best of this esoteric bunch, as follows:

- **To get the best students**. Even at the top, competition remains fierce. Lyon has to stake its claim on the affections of the brightest few hundred making their way to the *grandes écoles* by the reputation it establishes in the next few years. The counter-attractions of Paris will persist. But even as things are, 3,000 people compete for the 100 places at Lyon. Competence in some foreign language is required. French entrants are paid as junior civil servants.
- **Education through research**. Lyon offers three options — mathematics and *informatique*, science of matter (physics or chemistry) and life and Earth science. From the outset, students at Lyon will be plunged into research (and, during the first two years, taught to write and speak English). The requirements vary from one department to another, but most students spend half of the first three years of the four-year course on laboratory work and research. In the process of doing so, they normally acquire (from the University of Lyon) a first degree and, usually, the *diplôme d'études approfondies* (a necessary but not sufficient qualification for teaching at a university). Students at Lyon follow as a matter of course the laboratory-based *magistère* curriculum. They spend the last of their four years either preparing for a two- or three-year PhD course or in qualifying as fully fledged teachers in higher education (the process known as *Agrégation*. Aubert expects that 9 out of 10 will head towards a PhD.
- **Internationalization**. Like everybody else in France, Aubert is scheming to do his bit for the integration of Europe. One possibility is to recruit students from elsewhere to Lyon, perhaps as many as 50 each year. (The statutes allow foreign students to attend, and to pay no tuition fees, but they would not be paid or other-

Polytechnic Institutes

THREE relatively new institutions, in many ways intermediate in character between the *grandes écoles* and the national universities, have a particular influence. The best known is the Institut National Polytechnique de Grenoble, with roughly 3,000 students and an associated group of *grandes écoles* specializing in technical fields. There is also an Institut National Polytechnique at Toulouse and another in Lorraine.

All three were founded in the early 1970s as a means of stiffening higher technical education and lending coherence to its academic procedures. All are relatively small in terms of student numbers — between them the institutes have about 8,000 students. ☐

alt with as if they were French civil ts.) News of this scheme advertised yon's research network apparently ught 18 university people from else- ere in the European Communities and e European Free Trade Area to an nthusiastic meeting on 7 June. Further developments are awaited.

■ **Liberalization**. To guard against the danger that the *normaliens* will be over-narrowly educated, there is a scheme to mix students from the University of Lyon in with them. But there are no plans to follow, for example, the Massauchussetts Institute of Technology in introducing liberal studies of some kind to the curriculum.

■ **Research**. In the long run, the success of the Ecole Normale Supérieure de Lyon will hang on the reputation it acquires in research. There are almost 150 researchers already at work, two-thirds of them on the payrolls of either CNRS or INSERM. It is a guiding principle that there should be strong links between the school and the national research organization, with the result that two of the university's six laboratories are joint CNRS-Lyon institutes while the others are less formally linked to CNRS. Aubert is entirely content to think of using laboratories at Grenoble as well as Lyon both as research partners for Lyon's academics and as places at which the *école*'s students can follow their *magistère* programmes.

Much thought seems to have gone into the formulation of research programmes which, while having roots in traditional research, are conceptually innovative. For example, the people in *informatique* are concerned with the logic of parallel computers, the physics department with instabilities and order–disorder transitions in liquids.

So has the move indeed confirmed that it is possible to live, and to remain intellectually alive, outside Paris? Almost all the academics at Lyon have moved from Paris, although not from the old campus at Saint Cloud. Interestingly, three people (for this purpose a random sample in that their qualifications were that they speak English) had all used the opportunity of the move to Lyon to change the emphasis of their research. One common goal seems to be the creation of a distinctive line of enquiry, capable of catching national and international attention.

Those who have made the move are youngish people (and Aubert is proud of the average age of the faculty at Lyon). The reason is straightforward: as one explained, "the older people would not move". But even some of those who have done so acknowledge that there are ways in which "Paris is better". What they mean is that the extramural aspects of life in Lyon are not as varied as in the capital. That is perhaps something else on which Aubert should be working. ☐

UNIVERSITY OF PARIS

What's in a number?

Tell the average academic in Chicago, Manchester, Tokyo (and probably anywhere else outside France) that you teach at the University of Paris and he is likely to say "Ah!, the Sorbonne", perhaps adding that it is "the oldest university in the world, isn't it?". In fact, the Sorbonne ceased to exist in 1790 and was given to the University of Paris in 1808.

In troubled 1968, that was divided into 13 campuses or, more precisely, what is now the Académie de Paris comprises 13 universities — each one numbered. But the numbers have verbal equivalents. A researcher in physics might, for example, give his address as "Paris XI", but say he works at "Orsay". And the rector would call himself "*Professeur en Sorbonne*", not at Université de Paris I.

The confusion is even greater than it first appears. While three campuses might still call themselves "the Sorbonne", being on the original campus in the Latin Quarter, Paris I is known as "Tolbiac", Paris III as "Censier" and only Paris IV, the old faculty of letters and arts, still calls itself "Sorbonne". Meanwhile, the Université Pierre et Marie Curie is always known as Paris VI, while Paris VIII, set up as an experimental university after May 1968, is called "Vincennes", after its original location in the eastern suburbs, but is now at St Denis in the north.

Foreign academics would, however, be right in thinking that the University of Paris is, with Bologna, older than other European universities. It began as a theological school attached to the cathedral of Notre Dame. As the number of tutors grew, so did the variety of disciplines. Th whole became known as the university while tutors within a discipline groupe together as faculties.

The Sorbonne appeared in 1253, as theological school founded by Robert de Sorbon, and had a stormy history. It was opposed to the establishment of Jesuit orders in France and sided with the English against Joan of Arc. The Sorbonne was closed by Convention in 1790.

PARIS UNIVERSITY BY NUMBERS

Paris I	Pantheon-Sorbonne	"Tolbiac"
Paris II	(economic law, social sciences)	"Assas"
Paris III	Sorbonne Nouvelle	"Censier"
Paris IV	Sorbonne	—
Paris V	Rene Descartes	—
Paris VI	Pierre et Marie Curie	—
Paris VII	—	"Jussieu"
Paris VIII	Vincennes at Saint-Denis	—
Paris IX	Dauphine	—
Paris X	Nanterre	—
Paris XI	Paris-Sud	"Orsay"
Paris XII	Paris Val de Marne	"Creteil"
Paris XIII	—	"Villetaneuse"

Once integrated into the University of Paris, the "Sorbonne" refused to accept the teaching of new disciplines. Consequently, new universities were founded to teach sciences, humanities and the social sciences. Only after 1968 did universities throughout France become truly multidisciplinary. Today, in the first major reforms since the 1970s, eight new universities are planned, four in the Paris area.

With the growth of the university system as a whole, the numbers game has spread. In Montpellier, for example, there are "Montpellier I", "II" and "III", the first (teaching arts, literature and the humanities) also known as "Université de Paul Valéry".

Peter Coles

MAGISTERES

Gaining distinction by degrees

If universities can do little to select the students they teach, they can at seek to distinguish between those who leave with degrees. That is one function of the *magistère* programme, originally conceived by Jean-Pierre Chevènement in the early 1980s as a way of redressing the balance between the *grandes écoles* and the regular universities.

The result is a highly selective beefed-up diploma for university students called the *magistère*. First introduced in 1985 in 70 selected university departments, in a wide range of disciplines, the *magistère* is meant to prepare students for research or industrial careers no less promising than those of engineers from the *grandes écoles*.

Students enter the *magistère* after the first cycle (first two years) of university education, but must have a special mention in their diploma (DEUG). Because the *magistère* degree is not national, but awarded by the university, students almost always carry on their regular studies.

In the first year, students carry out supervised research at a state laboratory, one half day each week. In the second, they are working full-time in a research laboratory and, in France, are given priority for grants under the European Commission's Erasmus and Comett programmes to study abroad. Students from the Joseph Fourier University at Lyon last year went to the University of Sussex and others to Philips in Eindhoven, for example.

The ministry of education has no plans to expand the scheme at present; much will no doubt depend on an evaluation of the scheme completed by Dr Guy Aubert.

Peter Coles

GRENOBLE

Dissembling Alpine science city

If France were the Soviet Union, this Alpine city would be named Akademgorodok and everybody would be a little self-conscious about its strategic and economic importance. But the city's ambitions seem to lie somewhere between an upmarket ski resort and a smaller version of Paris (to which every provincial city aspires).

As if in that cause, the mayor of Grenoble, M. Carrignon, has in the past few weeks put the city on the national political map by advising members of the Republican (centrist) party to which he belongs to vote Socialist in the local elections so as to defeat the candidates of the extreme-right National Front. For his pains, Carrignon has been suspended from party membership.

But the nature of the place is apparent from the autoroute from Lyon, just over 100 km away. First, there are traditional conveyor belts carrying limestone from a scar on a miniature alp to the east to the cement factories in the valley of the Isère, then the unmistakable outline of a particle accelerator taking shape at about the point at which the exit signs read "*Polygone scientifique*". The circular hole is, of course, the European Synchrotron, the 6 GeV electron synchrotron designed to be a purpose-built source of X-rays and γ-rays when it is commissioned in 1993.

Grenoble has grown to be the place it is by a sequence of accidents, among which the first was its choice as the site for the basic research laboratory of the Commissariat à l'Energie Atomique (CEA). Another influence is more personal — that of Dr Louis Néel, who was still a member of the physics department at the University of Grenoble when he won a Nobel Prize (in 1970) for the discovery of antiferromagnetism. Néel laid the foundations for tempting to Grenoble the half a dozen CNRS laboratories, mostly centred on condensed-matter physics, that constitute the Polygone.

What more natural than that the city should also be the site of the thermal nuclear reactor which, under the name of Institut Laue-Langevin (ILL) is one of Europe's most prolific sources of thermal neutrons, used mostly for structural studies? The institute is jointly financed by France and West Germany, with smaller contributions from other members of the collaboration. Tucked away in the same *polygone* is a Max-Planck Institute of solid state physics, visible proof of West German commitment to the place.

Guessing at the numbers of scientific and technical people is more difficult at Grenoble than at, say, Novosibirsk, but CNRS is thought to employ about 700 qualified scientists here, CEA perhaps twice as many, while the less permanent populations of ILL and, eventually, of the European synchrotron laboratory, may account for a further 1,000. Then there is the university. Allowing for technicians and administrative people, direct employment in Grenoble's public laboratories is probably not far short of 15,000, a substantial proportion of the regional population, estimated at about 400,000.

But that, people are quick to say, overlooks the uncounted scientists and engineers working for the small technical enterprises that have sprung up (with the encouragement of the local authorities) at Grenoble, originally as suppliers of goods and services to the public laboratories, but which have now cultivated national and international ambitions. (The continuation of the autoroute from Lyon is plainly marked "Turin", but francophone Switzerland is even closer.)

Grenoble, in other words, is a living proof that life outside Paris is possible, and can even be rewarding. One benefit has been that the laboratory-based culture has been able to forge a strong relationship with the University of Grenoble, to their mutual benefit. CNRS laboratories regularly have academics among their staff members, while researchers teach courses at the university. (The incentive is not monetary, but the simple consideration that teaching experience counts as a plus when promotions are considered within CNRS.) Similarly, academics and people from the various laboratories sit on each others' assessment, promotion and strategic committees.

The academic links with research are plain to see. At the CNRS Laboratoire d'Etudes des Propriétes Electroniques des Solides, graduate students (from other universities as well as Grenoble) outnumber fully fledged researchers (a third of whom are Grenoble academics).

This fruitful symbiosis rests in part on bread-and-butter considerations: academics have access to more sophisticated equipment than university laboratories can usually provide, while laboratories can profit from the flexibility of the funds provided by the university towards the overhead costs of their academics' and their students' research costs.

But there is more to say than that. Dr Guy Aubert, head of the CNRS Service National des Champs Intenses until two years ago says that, in the physical sciences, CNRS offers the only mechanism in France by which the quality of the research people do can be sympathetically and objectively assessed. To link a university with the CNRS system provides it with a yardstick for the measurement of self-improvement and the means thereto.

It goes without saying that the symbiosis provides the laboratories with recruits who can hit the grounds running. Grenoble is both the originator and an enthusiastic exponent of the *magistère* programme (see opposite) by which students are introduced to research while still working for their first degrees, partly by being taught by researchers and partly by working in research laboratories or at computer consoles. The *polygone* has provided a sparkling new building in which to house the *magistère* students.

There is a general lesson to be learned from all this: CNRS laboratories can profoundly influence a nearby university. It is not just that CNRS laboratories can provide neighbouring academics with facilities for research that would otherwise be lacking (thereby making the universities attractive to potential teachers), but that they can also hope to mould both the university curriculum and the composition of its faculty.

But the symbiosis requires readiness on both sides. Other CNRS laboratories complain that they must take in graduate students from neighbouring universities over whose curriculum they have had virtually no influence. *Formation*, the academics say, "that's our responsibility". Then the symbiosis cannot work. Nor can it when there is no CNRS or INSERM laboratory within reasonable range.

It is no wonder that Grenoble (with Strasbourg, Orsay and Toulouse) has been singled out as one of the outstanding university centres in France. In reality, there are both the Polytechnic University, with 2,000 students, and the three campuses of the University of Grenoble dealing (by numbers) with science and technology (the Joseph Fourier University), with social sciences and with arts and literature. The city has a student population of 30,000.

Not everything in Grenoble is lovely. The worry about salaries, generally through CNRS, quickly bubbles to the surface. Working researchers say that industrial salaries are generally 50 per cent higher than their own. Most people acknowledge that it is worth paying something for the extra freedom and interest that their laboratories allow. But 50 per cent? Guy Aubert says that even laboratory chiefs are now regularly head-hunted — most expect to get one reasonable offer a year.

Promotion is also a constant worry. Becoming a *directeur de recherche* (a salary grade), requires not just a *concours*, but passing through the eye of a needle which appears ever to shrink in aperture.

Some at Grenoble are also alarmed at the shrinking proportion of CNRS's income available for the direct support of research. This relatively well-heeled group of laboratories is well placed to secure outside contracts, and does well, but is alarmed that external dependence may eventually threaten what it considers to be its core research. □

POLIS

uropean Los Angeles?

TPELLIER has all the makings of Los geles, but it has a long way to go. For time being, it differs by a fragment of a bstantial mediaeval city wall, the like of which has never been seen in California, while it is still possible to look at clear blue sky. But not for long, if the fastest-growing city in France can keep up the momentum, it may soon have enough road vehicles to generate a pall of smog.

Montpellier also has the distinction of beginning as a university town. There are 200,000 permanent residents, but 50,000 university students who come and go.

Apart from the abundant youthfulness, the city has gone in for three contrasting and inconsistent architectural gimmicks whose like cannot easily be seen elsewhere. First, the old centre of the city has been covered with a sheet of terrazzo as vast as St Peter's Square, from whose edges protrude the upper floors of modern buildings, most of them apparently triangular, which are founded on solid earth. (The centrepiece of the display is a theatre, the Comédie, in which few plays play.)

Second, there is a gigantic air-conditioned bunker a good 300 m long in a distant corner, said to have cost more than $2,000 million to build. It is a conference centre of the most modern kind, which provides the city with a car park and which is inhabited by the chamber of commerce and industry.

Third, there is the new home of the Conseil Général, the provincial government, constructed to a kind of athenian plan; there are half a dozen post-modern replicas of the portico of the Parthenon (with office suites behind) arranged in a semicircle and separated by a highway and an artificial river from the central office block, in glass and soft brown stone rising to a dozen stories. One Montpellier driver thinks the river a huge joke: "it would take a long time to get to the sea that way".

What is happening is that Montpellier believes it has a chance to become the most important city in the arc of the Mediterranean between Gibraltar and Marseilles. One picquant feature of the development is that the Socialist mayor of the city, Georges Frèche, and the Republican administration of the region, have sunk their political differences in this common cause.

Professor Roger Brunet, a geographer who is the director of the CNRS-financed "public interest group" RECLUS based at Montpellier, explains that there is a "launch-window" within which the city must somehow make its mark. ("RECLUS" stands for Réseau d'Etude des Changements dans lesLocalisations et des Unités Spatiales.)

The window opened in 1988, when the national government devolved to the departments certain responsibilities for education, planning and industrial development, and when Spain and Portugal joined the European Communities, enlarging the city's vision to the West. But growth had begun before that, in 1965, when IBM chose Montpellier for a manufacturing plant (now the chief source of employment after the three universities). Legend has it that somebody at IBM drew a line from the company's research laboratory at Nice to its import base at Bordeaux, and found Montpellier roughly half-way between.

With that beginning, Montpellier's ambition is to become a technopolis — one blessed with sun and sea (5 km to the south, past the airport and through a group of small factories). There are also the Cevennes mountains 10 km to the north. The other assets are the universities and the large group of research council laboratories (INRA is especially well established) scattered about the city. The city is also the main base of CIRAD, with responsibility for the cooperative development of tropical agriculture. The city and the region are setting out deliberately to exploit these assets, seeking to attract small and large enterprises in agriculture, pharmaceuticals and *informatique* to science parks physically designed to be contiguous with the laboratories in related fields.

Brunet, whose unit has general interests (it is using its expertise in cartography to compile an atlas of the world as well as of France, and has advanced the doctrine of territoriality as central to the present development of Europe) is a bystander, but an interested one. One of his projects is the conceptual design of a highway from Gibraltar to Constantinople, on which Montpellier would be a crucial staging-post.

So when will the launch-window close? Montpellier seems realistically to appreciate that the world has become competitive. Toulouse, the centre of the aerospace and much of the electronics industry, is just 100 km to the northwest. More irritating, Nîmes, 50 km in the other direction, and best known for its splendid Roman ampitheatre, is nursing ambitions to expand (as, indeed, it is). The window will close only if the plans now hatched fail to materialize. Nobody in Montpellier believes that will happen. ☐

NUMERACY

Industrial mathematics booms

INDUSTRY seems to have taken to the heavy-duty computer and the techniques of model-building with the enthusiasm with which the French public has embraced Minitel — the ubiquitous domestic videotext service originally devised as an electronic telephone directory, but now used for gathering general information, train times for example. The result is that industrial researchers have set out deliberately to recruit mathematicians to their cause.

The impetus is twofold. Simulation is often much cheaper and quicker than experimental investigation, vividly apparent in the use of simulation by aeroengine manufacturers in the design of turbine blades (whence, in part, the present concentration of interest in fluid dynamics). The recognition of that truth is not unique to France. But what may be is the recognition by French industry that mathematics is one of the country's strongest suits, and that mathematicians are not always as unwilling to bother with practical problems as their reputation suggests.

But how to capture that resource? Elf's Solaize laboratory believes it has found an effective way of doing this, on behalf of the company's research effort as a whole. J-P. Valentin is a regular mathematician on the laboratory's full-time payroll who is not merely allowed, but required, to spend half his time on academic work.

For the rest, Valentin's job is a mix of persuading researchers in the company's laboratories that many of their problems are often essentially mathematical problems and then of recruiting appropriate outside consultants who can help to solve them. He says that he would be unable to perform the second role if he were not himself a practising mathematician, able to mix with others on equal terms at conferences.

He describes the first function as if it were a kind of psychoanalysis. Most researchers, unaware of recent advances in mathematics, are slow and even unwilling to acknowledge that their problems may be mathematical in character. People used to making measurements with an experimental rig prefer to stick with what they know. He also claims to be a kind of midwife, recognizing that one research group may already have a solution to another's problem.

Valentin cites as an important technique in his business the organization of regular meetings at which Elf-sponsored PhD students (there are 120 of them scattered about the universities) present accounts of their research in the presence of their academic supervisors. These serve, he says, not just as ways of recruiting consultants, but as a powerful means of deepen the company's corporate culture of mathematics. ☐

NUCLEAR ENERGY

Reactors hit financial trouble

FRANCE's ambitious nuclear programme has wound up in a curious dispute over the ownership of the chief reactor-builder, Framatome, in whose shares its chief rival, the Compagnie Générale d'Electricité (CGE) has acquired a controlling stake. The government is in a quandary over whether to renationalize Framatome.

This is an unexpected but unavoidable outcome of the past two decades, during which France has striven to make its nuclear energy industry second only to that of the United States. But now, with a slump in oil prices and a worldwide wariness after the Chernobyl accident, it has found itself with knowhow nobody wants and more electricity than it needs.

The 53 reactors belonging to the nationalized EDF (Electricité de France) — mostly of the pressurized water type, but including one fast-breeder reactor — supply 75 per cent of the country's 342,000 million kilowatt-hours of electricity production. With very few coal, gas and oil resources, France began strengthening its nuclear capability during the 1970s oil crisis. Successive governments continued the expansion policy, supported by a generally enthusiastic public.

France also has stakes in all aspects of the generation cycle. Cogéma, the industrial subsidiary of the Commissariat à l'Energie Atomique (CEA), the state research agency, has almost cornered the world market in irradiated fuel reprocessing at its La Hague plant on the coast of Normandy, on the English Channel, with a capacity intended to be 1,600 tonnes a year by 1992 and as much as 40,000 tonnes by 2000.

Just as France's own construction programme matured, the Chernobyl accident led other countries to freeze their construction programmes, leaving France's two major manufacturers of nuclear technology, Framatome and CGE, with little hope of critical export orders. The situation was made worse by a saturated home market. In the 1980s, ten new reactors were commissioned to meet an expected demand that never materialized. And a new generation of fast-breeder reactors was planned to follow the experimental 1,200 MW Superphénix plant at Creys-Malville on the Swiss border. But soaring costs, unreliability, cheap uranium and national overproduction led this programme to be shelved.

To add to the slump in enthusiasm for nuclear reactor construction, doubts have now been raised about the safety of existing reactors. Earlier this year, it was revealed that the wrong bolts had been used in maintenance on safety valves at the Gravelines site on the English Channel coast near the Belgian border. A report commissioned by EDF revealed similar incidents elsewhere and that the risk of a major accident in the next 20 years at one of the French reactors could be much higher than previous estimates. There are six 1,300 MW reactors on the Gravelines site; even the remote threat of an accident has made France's neighbours twitchy.

But the need to replace old reactors, combined with expectations that industrial growth will increase energy demand in the next decade, means that the reactor construction programme is not dead. Only last week, EDF promised that a new 1,400 MW reactor at Civaux would get the go-ahead next year after long delays.

The prospect of renewed growth has put Framatome and CGE at the heart of a political storm. Last month, CGE bought out shares in Framatome held by Dumez, giving it a 52 per cent of the company, a controlling stake. The government feels uncomfortable with its 45 per cent minority share (35 per cent CEA and 10 per cent EDF) and is now wondering whether to intervene by nationalizing Framatome again, so as to gain control over little-valued but potentially critical expertise.

CGE, privatized in 1987, is against such a move and, in any case, nationalization is likely to run up against a ruling of the present government guaranteeing neither privatizations nor nationalizations of industries in the current mandate.

Peter Coles

Science parks multiply

RESEARCH may not immediately make nations prosperous, but it can do wonders for the prosperity of cities. That seems to be the principle on which several municipal governments are now working, from Lille and Rennes in the north and Lyon in the centre to Montpellier in the southwest.

So science parks are multiplying. At Montpellier, there are no fewer than three, conceptually centred on the city's intellectual assets.

The presence of IBM has stimulated the city, in the 1980s, to persuade small companies in *informatique*, robotics and artificial intelligence to what it calls its Parc du Millénaire.

Agropolis is the name for the similar effort in agronomy and agricultural biotechnology, the core of which is the complex of government laboratories with interests in food and agriculture, between them employing an estimated 1,800 people.

There is also what Montpellier calls its Euromedicine park, to which the city has attracted more than 20 businesses and research laboratories in the fields of pharmaceuticals and medical instrumentation.

What stands out from these endeavours is that the public laboratories are more than merely compliant, they are encouraging. It is public policy not only that France should increasingly rely on technology for its prosperity, but that neighbours with interests in research may be a source of research contracts, perhaps even of better jobs. ☐

INDUSTRIAL RESEARCH

Getting back to business

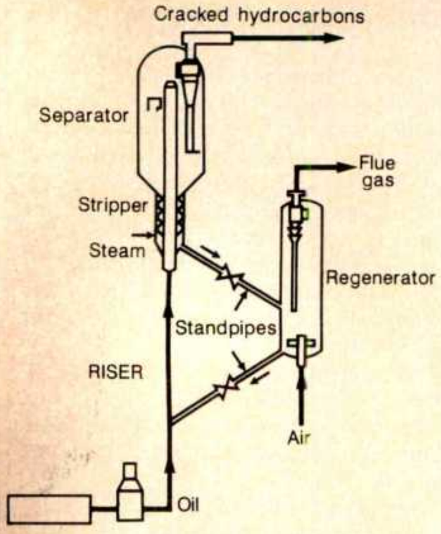
CRUDE oil refiners in France have been driven into a corner — and into research — by the nuclear energy industry, and by the collapse in the market for heating oil. Elf-Aquitaine, which operates three refineries in France, says that the annual throughput of crude has fallen from 100 million tonnes a year to only 80 million tonnes, and demand for heating oil has fallen even more drastically.

The problem is how to win a greater yield of saleable products, notably petrol (gasoline, otherwise *essence*) by the intelligent cracking of crude. The company claims to be a pioneer in the automatic control of refinery operations. Now, at its research laboratory at Solaize, south of Lyon, it has a team of eight people perfecting the operating parameters of catalytic petroleum crackers, believing that understanding will lead to a greater yield of lighter fractions.

The research programme has been a joint effort by Elf and CNRS since 1956. A group of four CNRS researchers have been seconded to the Elf laboratory. When their project finishes two years from now, the CNRS people will return to their laboratories not merely with a better appreciation of industrial problems, but with an expertise in the operation of large pilot plants. Nobody seems alarmed that they will also be the better able to advise Elf's competitors.

The problem of optimizing the design and operating parameters of a cat cracker is complex. J.R. Bernard, the Elf researcher who leads the group, likens it to the problems that arise in the design of nuclear fuel rods and the fixing of operating parameters.

The catalysts are zeolites, used in the form of 60-μm grains which give the material the appearance of a brownish-pink powder. Standard practice entails the use of heavier distillation fraction of the crude to support an upward-moving fluidized bed of catalyst in what is inevitably called a 'riser'. The cracking process appears to require as little as 2 seconds, which is a pointer to the speed at which materials are circulated through the plant. Petroleum products are stripped from the catalyst by steam and the catalyst recycled



Catalytic cracking plant Elf style.

through an air-driven regenerator whose chief function is to remove solid carbon, or 'coke', from the catalyst (see figure).

In the drive for increased gasoline output, schemes for the hydrogenation of heavier crude fractions are being canvassed, further complicating the optimization of cracking plants.

At Solaize, the joint Elf-CNRS team has been forced back to basics, fitting a pilot-plant housed in a hangar-like building with instruments and using radioactive tracers for tracking the movement of zeolite through its cycle. The objective is to refine a mathematical model of a cracking plant which is being elaborated, with the objective of fixing the parameters of more efficient cracking plants linked with hydrogenation units. The same model will eventually be the basis of programs for the automated control of refinery operations.

Bernard enthuses about the niceties of the operation of standard cracking plants which the study has brought to light. Even questions such as the effect of the riser walls on the movement of zeolite had previously gone unanswered, he says. That there should be friction had been expected, but the velocity profiles across the riser that have now been measured, and the patchiness of zeolite concentration across the riser, were not expected.

Nobody at Solaize is able to say what the economic benefits of the programme will be. Rather, the assumption seems to be that a business which depends on the successful operation of machines such as cat crackers had better understand them in as much detail as it can.

Elf is not alone among French companies in having taken a deliberate decision in the past decade to spend more of its effort on basic research. Claude Jablon, corporate director of research, says that the company (which differs from more conventional oil companies in its strong interest in chemicals and pharmaceuticals) employs 4,500 people in research laboratories scattered through France, elsewhere in Western Europe and in North America. Of this effort, engaging some 4 per cent of the company's total staff, he reckons that 10 per cent is devoted to projects that will yield benefits only in the long term.

Just where the project to develop better asphalt fits in this is not clear. The company appears to have something of a success with a material called 'Styrelf', selling 300,000 tonnes a year of it. The idea is that hydrocarbon molecules of regular asphalt (non-volatile refinery fractions) have been covalently linked with styrene to form a kind of three-dimensional molecular network in which road metal (graded granite fragments) for highway surfaces can be embedded. Energy is being spent on the materials testing of various compositions of road surfaces, but, in the absence of a full understanding of why highway surfaces wear out or become distorted, faith is required to assure highway authorities that polymeric asphalt will last longer. ☐

SPACE

Is there profit as well as pride?

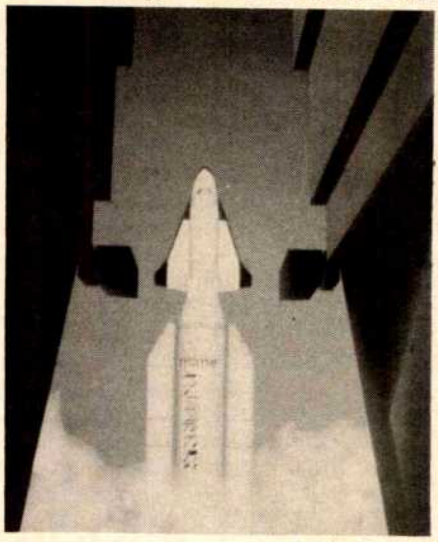
Over the past 25 years, France has emerged as the European leader in space technology, continuing to push forward and take expensive risks while its neighbours got cold feet. This fits with the high profile given to technology.

The academic elite graduating from the *grandes écoles* are called 'engineers', and electronic gadgetry sprouts regularly in Paris, from video in the metro stations to cable television. The Minitel telephone-linked interactive videotext system is the most successful in the world and France's high-speed trains (TGV) and modern metro systems are often cited as models elsewhere. High technology is seen as the key to economic survival in the next century.

France has had its own space programme since 1962, when the Centre National d'Etudes Spatiales, the national space agency, was founded. In 1975 it abandoned its own domestic rocket programme to cofound the 13-member European Space Agency (ESA).

Now, France contributes almost a third of ESA's $1,700 million budget, ahead of Germany and Italy and four times as much as Britain. It is no accident that ESA headquarters are in Paris, that the European commercial launch consortium — Arianespace with 50 per cent of the world market — is based in France, or that the first European cosmonaut was a Frenchman.

Always an enthusiastic partner in ESA, France pulled out all the stops at the 1987 ministerial meeting in The Hague, at which the agency's long-term plan was approved. The Hermes space plane developed by CNES was chosen to service the Columbus platform, intended as Europe's contribution to the US Freedom space station, despite criticism from Britain that the technology was out of date and that the plane was too small.


ESA

Ariane 5 will launch Hermes — both products of French initiatives.

But the price of the acceptance of Hermes is that France has to foot 45 per cent of the bill, a cool FF597 million in 1990. Furthermore, Hermes depends on a new generation of heavy-lift launcher, Ariane 5, to get into space and this, too, is 45 per cent French, with costs to France running at FF1,812 million this year.

But while Britain decided that the Hermes and Ariane 5 package was simply too expensive and dropped out, France found it had money to spare to boost its domestic space programme. In the 1990 civil research budget, CNES received FF7,187 million — almost 16 per cent of the entire budget and 11 per cent more than 1989.

Of this, FF352 million will go towards a fourth generation of the SPOT commercial remote-sensing satellite owned by CNES, which will have cost an estimated FF2,500 million by the time it is launched in 1995. Although SPOT loses money, its freely available high-resolution photographs have proved a thorn in the side of superpower defence departments and a way to vaunt France's prowess.

As European space technology has a distinctive French tinge, it is fitting that the new director of ESA should be a Frenchman, Jean-Marie Luton, who came from CNES. And with some tough negotiating ahead as the full costs of Hermes and Ariane 5 emerge, Luton will need all the backing he can get. **Peter Coles**

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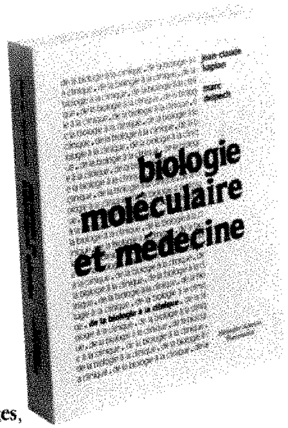

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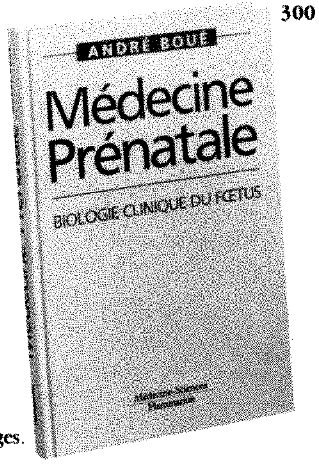

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The arid–humid transition in the Sahara and the Sahel during the last deglaciation

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At the time of the Last Glacial Maximum, the Sahara and Sahel regions of North Africa were extremely dry. New records of rainfall show that, during the subsequent deglaciation, the transition from arid to humid conditions in these regions occurred synchronously in two main steps. Comparison with other records of palaeoclimate in Europe and the North Atlantic Ocean shows that certain common factors controlled changes in ocean and atmosphere dynamics during the deglaciation.

THE structure and timing of the last deglaciation is now well documented by numerous oceanic records and continental sequences between middle and high latitudes[1]. The last glacial-interglacial transition is not a simple linear response to astronomical forcing, and the two-step deglaciation model is now supported by detailed evidence[2-7]. Results of general circulation models suggest that climate change in the regions around the North Atlantic Ocean has been influenced greatly by changes in the northern Atlantic Ocean dynamics associated with the meltwater history of the Laurentide Ice Sheet. The effects of cold melt water may explain much of the nonlinear response of climate to astronomical forcing at high latitudes, but they do not provide a complete explanation for the sequence of change observed over North Africa[8].

In the Sahara and the Sahel, the Last Glacial Maximum (LGM) coincides with an episode of extreme aridity, whereas humid conditions prevailed during the early and mid-Holocene[9]. From simulated hydrological budgets, precipitation over the Sahel at 9 kyr BP (before present) was estimated at 125-130% in comparison with today[10]. The last deglaciation period thus corresponds to a major arid-to-humid transition (AHT).

Sediment cores of several tropical lakes span this transition[9]. In western sub-Saharan Africa (~5-15° N) and in the Tibesti mountains, the return of humid conditions before 12 kyr is well known from palaeolake and river-terrace studies[11-16], detailed palynological offshore profiles[17], and increases in the discharge of large rivers to the ocean[18]. A sudden drop in the level of several tropical lakes around 11-10 kyr has been tentatively linked to changes in the ocean circulation[19]. Large inputs of melt water from the Laurentide Ice Sheet in the northern North Atlantic would have reduced the North Atlantic Deep Water (NADW) formation during the Younger Dryas event[20], slowing down the North Atlantic's conveyor-belt circulation and inducing anomalies of sea surface temperature (SST)[19,21]. A colder North Atlantic may have stabilized the lower atmosphere and helped block the advection of moisture over North Africa[8] and may be responsible for a weaker monsoon rainfall over the northern tropics[19]. But few results ascertained from African lands provide a sufficiently defined chronology of the late Glacial to allow for high-resolution correlations with the oceans. Also, very little is known north of 15° N before 10-9 kyr. Because there are no continuous records from the early Holocene in the northern Sahara, the concept of a progressive reinstatement of a humid climate between the Equator and the Maghreb from 13 to 6 kyr has been advanced[22,23]. This hypothesis was followed by several authors[24,25], and developed in terms of general atmospheric circulation[23,26]. We show here that the re-establishment of wet conditions occurred before 12 kyr over both the northern and the southern margins of the Sahara, and that the climate evolution of the Sahel has been more complex than previously thought.

Although local hydrological factors may largely influence their water level and water chemistry[27,28], groundwater-fed palaeolakes of the Sahara and the Sahel can be very sensitive climate indicators provided that: (1) the extent of the recharge zone of the aquifer is relatively restricted to allow climate reconstruction at a local scale; (2) the response time to rainfall events is negligible; (3) the chemical and, in favourable cases, isotope characteristics of the ground water are known; (4) variations in hydrological conditions are not masked by evaporite dissolution; and (5) the hydrology is not influenced by sea level fluctuations. These conditions are met in two closed depressions selected from numerous sites studied in the PALHYDAF project[14,29], in the northern Sahara (Site 1, Sebkha Mellala, Algeria) and in the Sahel (Site 2, playa of Bougdouma, Niger), respectively (Fig. 1). Cores from the centre of these depressions provide continuous profiles from the late Glacial to the Holocene through sediments of aquatic origin (Fig. 1).

Crucial problems raised in ^{14}C dating in such environments, especially ageing by groundwater supplies and recrystallization due to water-table fluctuations, have been thoroughly discussed[15,29,30]. For carbonate dating, authigeny and the absence of recrystallization were verified by scanning electron microscopy (SEM) and the authigenic fraction (generally $<80\ \mu m$) was separated by sieving. The ^{14}C dates (conventional and accelerator mass spectrometry (AMS), uncorrected for secular variations) are regarded as valid when the ^{13}C content indicates that the total dissolved inorganic carbon (TDIC) of the water body was in (or close to) equilibrium with the atmospheric CO_2. In a given section, interpolated and extrapolated ages were calculated in intervals of similar lithofacies.

Three types of palaeohydrological indicators are considered: lithofacies and mineralogy, stable isotopes on carbonates, and biological remains. The stable isotope (^{13}C, ^{18}O) contents on authigenic inorganic carbonates are normalized with respect to calcite using the proportions, determined by X-ray diffractometry (XRD), of aragonite and dolomite and applying the proper isotope fractionation factors[31]. Carbonates from temperate lakes with very high turnover rates may reveal variations in the heavy isotope content of rains that are temperature-dependent[32-35]. In seasonally dry climates, the enrichment of the groundwater supply in heavy isotopes by evaporation is the major factor controlling the ^{18}O content[36]. In both cases, the crystallization temperature is not an important factor because the temperature dependence $\Delta\delta^{18}O_{carb}/\Delta T$ is much smaller than the variation in $\Delta\delta^{18}O_{carb}/\Delta\delta^{18}O_{water}$. In most North African lakes, the ^{18}O content of carbonates must be regarded as an indicator of the ratio of groundwater input to evaporation. The ^{13}C content gives further information on environmental conditions[15,28,30] such as water mixing, residence time and the intensity of biogenic production. Among the biological remains, attention is given to diatoms found in the Sahel sediments which allow chemical variables to be inferred from stratigraphic profiles by using calibration functions established from 200 modern African samples (refs 37, 38 and F.G., unpublished data).

Northern Sahara records: Sebkha Mellala, Algeria

Site 1 (32°11′ N, 5°12′ E, ~128 m.a.s.l. (metres above sea level); Fig. 1) is a closed depression, wind deflated through Cretaceous and Tertiary limestones and sandstones. It is hyperarid, with rare precipitation of northern origin (mean rainfall 42 mm yr^{-1}; mean evaporation 2,000 mm yr^{-1})[39]. The basin, dry today, is a discharge zone of the large confined aquifer of the 'Continental Intercalaire' which has its source mainly in the M'zab heights ~150 km to the west, but also in the Saharan Atlas mountains (Fig. 1)[40,41]. This deep (−1,000 m) aquifer supplies the local unconfined aquifer (water table at ~−6 m) through upward leakage of fossil water[40]. The basin has thus registered, with no lag, changes in precipitation in the recharge zones of the aquifer of the 'Continental Intercalaire'. The ground water, of $\mathrm{Na^+}$-$\mathrm{Mg^{2+}}$ or $\mathrm{Na^+}$-$\mathrm{Ca^{2+}}$ and $\mathrm{SO_4^{2-}}$-$\mathrm{Cl^-}$ type[41], is slightly saline (2.5-3.5‰). The stable isotope contents are −8.8‰ relative to standard mean ocean water (SMOW) for ^{18}O of the water and −11.5 to −9.1‰ relative to the PDB standard for ^{13}C of the TDIC. The ^{14}C content of the TDIC is below the detection limit[40,41]. The dimension of the aquifer suggests that these values apply to the Holocene.

The depression is filled by 8 m of sebkha and lake sediments (Fig. 1) that lie on the bedrock. Detailed results on the sedimentology, mineralogy, stable isotopes on inorganic and biogenic carbonates, element geochemistry of ostracod valves, and palaeobiology are discussed elsewhere[42,43]. The mineralogy and stable isotope variations are shown in Fig. 2. Radiocarbon ages on carbonates are accompanied by ^{13}C values > −4‰, far above that of the TDIC of the aquifer. Changes in ^{18}O content (−7.29 to +4.83‰) indicate large fluctuations in the ratio of groundwater influx to evaporation.

An arid climate is first registered (~15 kyr BP) by the dominance of aeolian sand, in association with gypsum which indicates a discrete and probably continuous discharge of $\mathrm{SO_4^{2-}}$-rich ground water. The overlying sediments are mainly composed of chemically precipitated minerals. The absence of detrital material indicates that the supply of local runoff is negligible, and suggests that the dunes of the Great Eastern Erg (Fig. 1) were colonized by vegetation. From 14.5 to 11 kyr, gypsum precipitation predominated in a saline water body, although minor amounts of authigenic carbonates allow AMS dating and stable isotope analyses. The filling of the depression at ~14.5 kyr, is recorded by a drastic drop in δ^{18}O (+0.31 to −7.21‰) and by high values of the ratio of groundwater input to evaporation at 12.8-12.7 and at ~12 kyr. The interval 10.6-9.3 kyr contains mostly gypsum and dolomite. SEM observations show that dolomite crystals are idiomorphic, and thus authigenic. The primary or early diagenetic dolomite represents the thermodynamically stable carbonate species in the Sebkha Mellala deposits after calcium removal through gypsum precipitation has increased the Mg/Ca initial ratio of 0.7 (ref. 41) in the highly concentrated residual solutions[44]. This disordered ($\mathrm{Mg_{44}}$, $\mathrm{Ca_{56}}$) dolomite is thus considered to be a post-gypsum evaporite. The interval 10.6-10.3 kyr has the highest δ^{18}O values (+1.72 to +4.83‰) in the whole section, and is considered to be the period of maximum local aridity. Arid conditions ended suddenly after

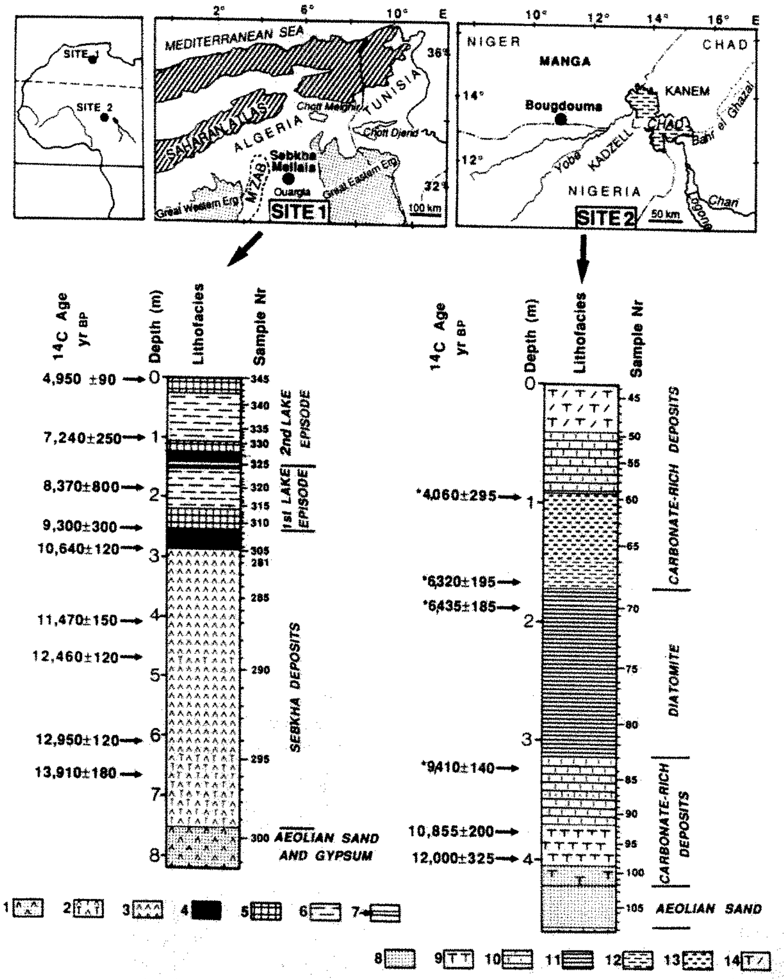

FIG. 1 Location map of the sites and simplified stratigraphy of the cores. Asterisks indicate radiocarbon ages of organic matter, others are ages of authigenic inorganic carbonate. Lithofacies: 1, aeolian sand and gypsum: 2, massive carbonate-rich gypsum deposit; 3, massive carbonate-poor gypsum deposit: 4, laminated gypsum and carbonates: 5, laminated carbonates free of mollusc shells: 6, alternation of shell beds and of carbonate and gypsum layers: 7, palaeosoil: 8, aeolian sand: 9, sandy carbonates: 10, diatomaceous carbonates: 11, diatomite-free carbonate: 12, calcareous clay: 13, calcareous sand: 14, carbonates and evaporites.

~9.3 kyr allowing the establishment of a lake, which was maintained until ~7.5 kyr. The humid episode (9.3-7.5 kyr) is registered by an abrupt decrease in $\delta^{18}O$ (+4.51 to −4.76‰), abrupt mineralogical changes and by the appearance of biological remains. Gypsum is still present, but the dilution of the solutions is indicated by the precipitation of Mg-calcite (associated with aragonite) and of calcite, which successively predominate in the sediments. The biological community reflects a saline shallow, but permanent, water body. No diatoms were found, possibly in response to secondary dissolution. The co-occurrence of freshwater organisms (such as the gastropod *Hydrobia* sp.) and of species able to tolerate marine-like conditions (such as the bivalve *Cerastoderma glaucum*, the foraminifera *Ammonia becarii, Triloculina* sp.) or hypersaline conditions (such as the gastropod *Potamides conicus*, the ostracod *Cyprideis* gr. *torosa*, or the charophyte *Lamprothamnium* cf. *papulosum*) indicates large short-term fluctuations in salinity, a common character of Holocene lakes of the northern Sahara[27,28]. The lake dried up at ~7.5 kyr, as recorded by a palaeosoil bracketed by dolomite-rich deposits, before the commencement of a new cycle of carbonate sedimentation dated from ~7.2 to 4.9 kyr. The top of the sequence is wind deflated.

We therefore identify two major steps in the transition from arid to humid conditions at ~14.5-12 (AHT1a) and 9.3-8.4 (AHT1b) kyr, the first one corresponding to several dry-wet oscillations. A third, smaller, step occurred at 7.5-7.2 kyr (AHT1c).

The Sahel records: Bougdouma, Niger

Site 2 (13°19′ N, 11°40′ E, 337 m.a.s.l; Fig. 1) lies in the Sahelian zone where most of the precipitation is due to summer squalls (75%) and monsoon rainfall[45] (mean rainfall ~250 mm yr; mean evaporation 2,000 mm yr^{-1} (ref. 46). The Manga plateau (Fig. 1) is the eroded surface of an old erg, probably of early Pleistocene age, and lies between ~400 (northwest) and 300 (southeast) m.a.s.l. over 120 km. The low gradient and the high vertical permeability of the aeolian sand do not allow for the development of a surface-drainage network. The unconfined aquifer is exclusively recharged by local rainfall[47], and crops out in numerous closed interdunal depressions. This was also the case during the late Quaternary, contrary to previous opinion[48] and the results of a related climate simulation[49], according to which the plateau was submerged by Lake Chad (present altitude 282 m.a.s.l.)[15,50]. Presently, the deepest depressions are occupied by small water bodies ranging from freshwater to hyperalkaline swamps, or playas with trona crusts (such as Site 2). In January 1989, ground water (Na^+-HCO_3^- type, conductivity = 5,800 μS cm^{-1}, pH 8.41, $\delta^{18}O = -4.01$‰ relative to SMOW, $\delta^{13}C \approx 0.0$‰ relative to PDB) was found 2.80 m below the surface.

The late Glacial climate evolution is deduced from a 4.58-m core (Fig. 1) whose detailed analyses are given elsewhere[51]. Figure 3 shows the stable isotope and diatom data. The diatom flora, of fresh to mesosaline water of the Na^+-CO_3^{2-} type, from a neighbouring core have been previously discussed[52]. Sudden peaks of the planktonic form *Cyclotella stelligera* var. *glomerata* reflect abrupt influxes of dilute ground water[52]. High absolute diatom contents coincide with high percentages of freshwater planktonic species (mainly *Melosira ambigua, M. granulata* and varieties), and indicate stages of relatively deep and dilute waters. The diatom influx was lower during phases of shallow saline waters. Very low diatom contents associated with bad frustule preservation (between ~12 and 11 kyr, for example) are probably the result of partial dissolution under fluctuating, ephemeral saline conditions[52].

At the base of the core, a calcareous diatomite could not be reliably dated, even by AMS, because of obvious traces of aquifer diagenesis on the carbonates, and despite the presence of a few millimetre-sized charcoals which are highly vulnerable to impregnation by hydrosoluble organic matter[51]. An episode more arid than today is registered by an overlying pure aeolian sand.

A sudden groundwater influx occurred before 12 kyr, and led to shallow saline conditions, with aeolian sand still predominant in the sediments. A second isotopic dilution is registered at ~12 kyr, and coincides with the base of a calcite-rich (15-50% of bulk sediment[51]) sedimentary episode. A water body, possibly ephemeral as suggested by the presence of the ostracod *Heterocypris salinus* and the bad preservation of diatom valves, persisted up to ~11 kyr under highly evaporative conditions. A new rise in the aquifer is recorded at 10.9-10.6 kyr. The isotope concentration then increased and reached the maximum value observed in the section at ~10.3 kyr. Diatoms show that generally dilute open-water conditions persisted up to that date, however, probably because of seepage of heavy solutions through the permeable lake bottom which may have regulated the salinity, as in the case of Lake Chad today[46,53]. At ~10 kyr, ~300 years later than the maximum isotope concentration, diatoms indicate a brief episode of very shallow saline-alkaline conditions. This is attributed to the leaching, by local rainfall or groundwater influx, of a highly soluble Na^+ carbonate crust deposited during a short but marked dry phase between ~10.3

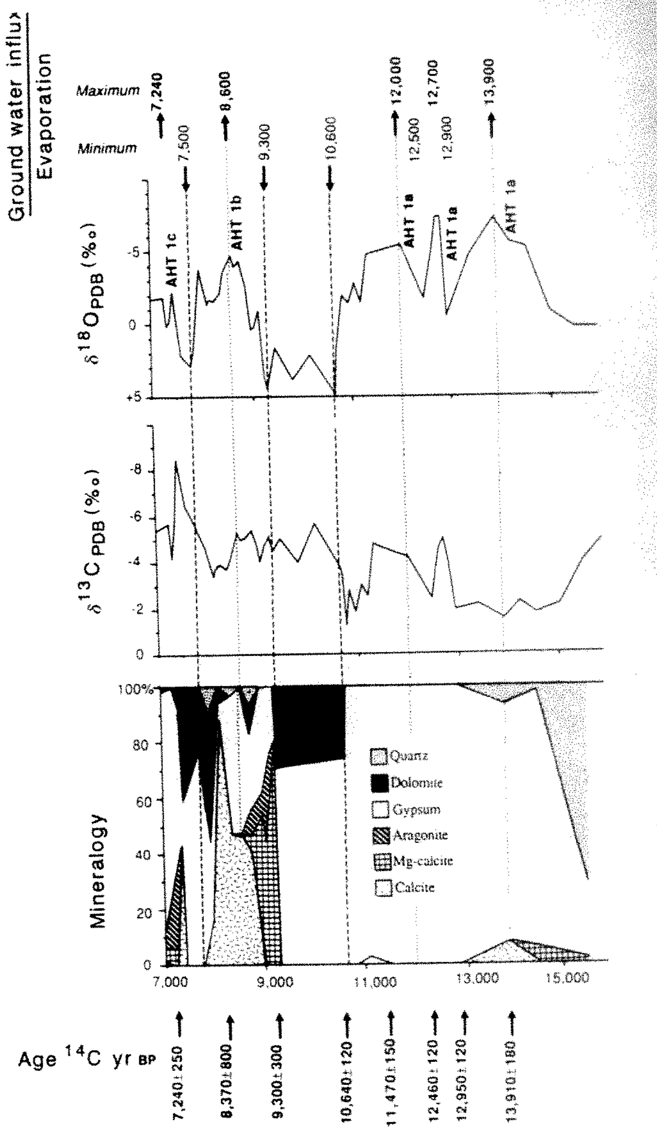

FIG. 2 Palaeohydrological indicators plotted against ^{14}C ages (16–7 kyr BP) for Site 1 (Sebkha Mellala, Algeria). Bottom, Mineralogy (XRD peak areas) of bulk sediment (biological remains excluded). Middle and top, Stable isotope contents of inorganic carbonates (fraction <80 μm) normalized to calcite[31] ($\delta^{13}C$(‰) = $[(^{13}C/^{12}C)_{sample}/(^{13}C/^{12}C)_{standard}] - 1$; $\delta^{18}O$ defined similarly). Arrows at the top mark the direction of the major hydrological events.

and 10 kyr. From ~9.7 kyr, the establishment of an early Holocene freshwater lake is registered first by a sharp peak of *Cyclotella stelligera* var. *glomerata*, increased diatom content and salt dilution, and then by a sudden stable isotope decrease. The hydrological turnover peaked at ~9.2 kyr when the calcite sedimentation was replaced by the accumulation of a diatomite almost free of carbonate. The freshwater lake persisted until ~8-7 kyr when the salinity began to fluctuate. Calcite reappears in the sediments at ~6.4 kyr. The top of the sequence records several hydrological fluctuations, including a sudden rise of the aquifer at ~4 kyr.

During the last deglaciation, two major phases of hydrological changes, ≤12 kyr (AHT1a) and ~9.7-9.2 kyr (AHT1b), are recorded in the Bougdouma core, bracketing a period of generally high evaporative conditions. During the Younger Dryas, however, there was a brief but clear humid phase dated from ~10.7-10.3 kyr. This is also recorded in individual interdunal depressions along the northwestern margin of Lake Chad[15,52] and was of regional extent.

Timing of the major climate events

The hydrological settings of our selected sites makes their hydrology and sedimentology dependent on the local rainfall. The two lacustrine records illustrate the extreme rapidity of the climate fluctuations that occurred over the Sahara and the Sahel during the last major arid-humid transition. The shifts from arid to humid conditions occurred in a few centuries, possibly in a few decades. Abrupt climate changes occurred at both sites at >12 (AHT1a) and at ~9.3 (AHT1b) kyr, with a reversal (H-A) centred on 10.5-10 kyr (Fig. 4). Both the northern and southern margins of the desert have thus experienced periods of excess input before 12 kyr, and probably since ~14 kyr. Our

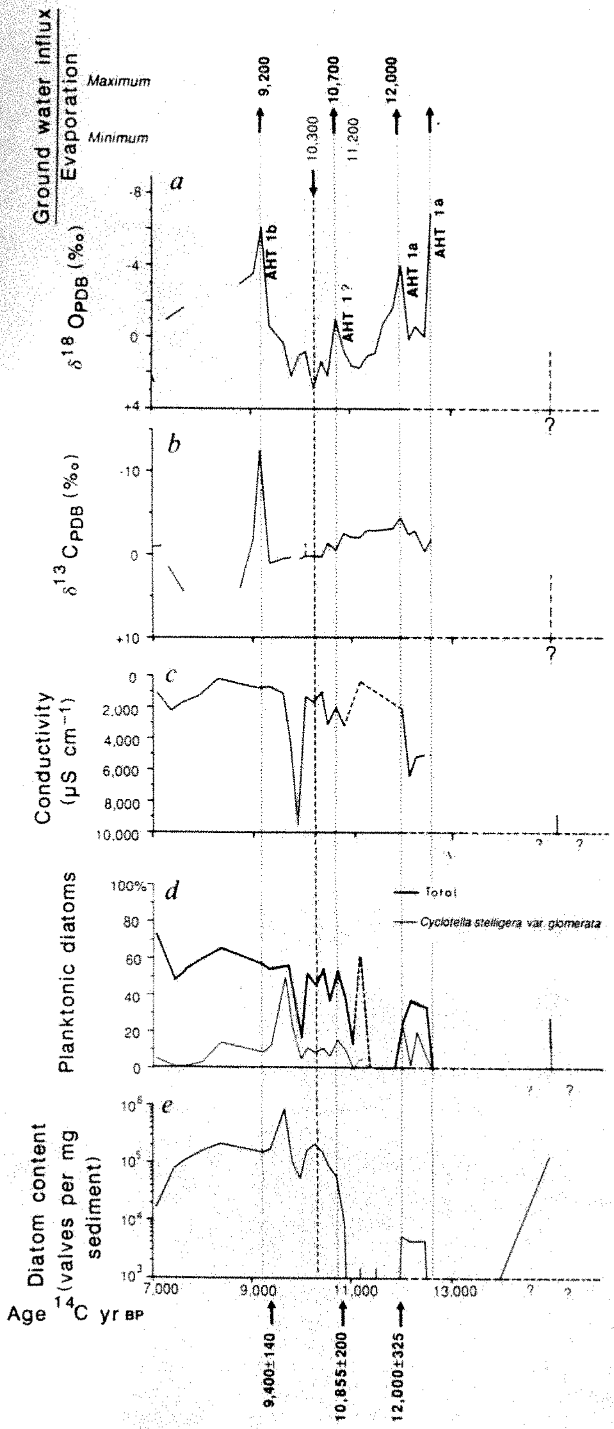

FIG. 3 Palaeohydrological indicators plotted against ^{14}C ages (16-7 kyr BP) for Site 2 (Bougdouma, South Niger). *a*, *b*, Stable isotope contents or inorganic carbonates (fraction <80 μm). *c*, Diatom-inferred water conductivity based on transfer function (refs 37, 38, and F.G. unpublished data). *d*, Relative percentages of planktonic diatoms. *e*, Sediment diatom content. The results in *c* and *d* are uncertain between 12 and 11 kyr owing to partial selective dissolution. Arrows at the top mark the direction of the major hydrological events.

data agree with previous data documenting the presence of early Holocene lakes in the northern Sahara[27,28,54], and do not support the hypothesis of a progressive re-establishment of wet conditions from the Equator to the Maghreb between 13 and 6 kyr[22,23].

Relationships to global climate change

Although stratigraphic uncertainties still exist, a comparison between our African sequences and the Atlantic and European records (Fig. 4) suggests that common factors have controlled the major changes in the ocean and atmosphere dynamics at low and high latitudes during the last deglaciation. The principal steps of the arid-humid transition, AHT1a and AHT1b (Figs. 4*c–e*), seem to have occurred at the same time as two intervals of rapid sea level rise deduced from coral-reef drilling and described as meltwater pulses 1A (12 kyr) and 1B (9.5 kyr)[6] (Fig. 4*h*). They coincide with the latter part of Termination 1A, and with Termination 1B as defined from deep-sea ^{18}O records[2-5] (Fig. 4*f*, *g*). Our chronology is also consistent with changes in the mean annual air temperature in Britain (reconstructed using beetle remains) which show two abrupt rises at ~13–12.2 kyr and ~10.2–9 kyr[55] (Fig. 4*b*), and with the European subdivisions based on cold-warm oscillations[56,57,34,35] (Fig. 4*a*). In both African sites, the phase of maximum aridity falls in the cold European Younger Dryas chronozone.

In the northern Sahara, the beginning of the first step (AHT1a) at ~14.5 kyr corresponds to the first signal of Termination 1A in the North Atlantic[2,20] and to the first appearances of hydro-thermophilous species in the pollen records from southwestern Europe[58]. The phase of maximum aridity started at ~10.6 kyr during the first part of the European Younger Dryas, and ended at ~9.3 kyr when warm conditions were fully re-established in Europe (Fig. 4*b*, *c*). These changes in rainfall, of Mediterranean or Atlantic origin, may have also influenced the vegetation of southwestern Europe.

In the Sahel, the interval 12–10 kyr corresponds with generally high evaporative conditions (Fig. 4*d*). The maximum aridity recorded at Site 2 between 10.3 and 10 kyr seems to match a maximum in the ^{18}O content of foraminifera and a minimum

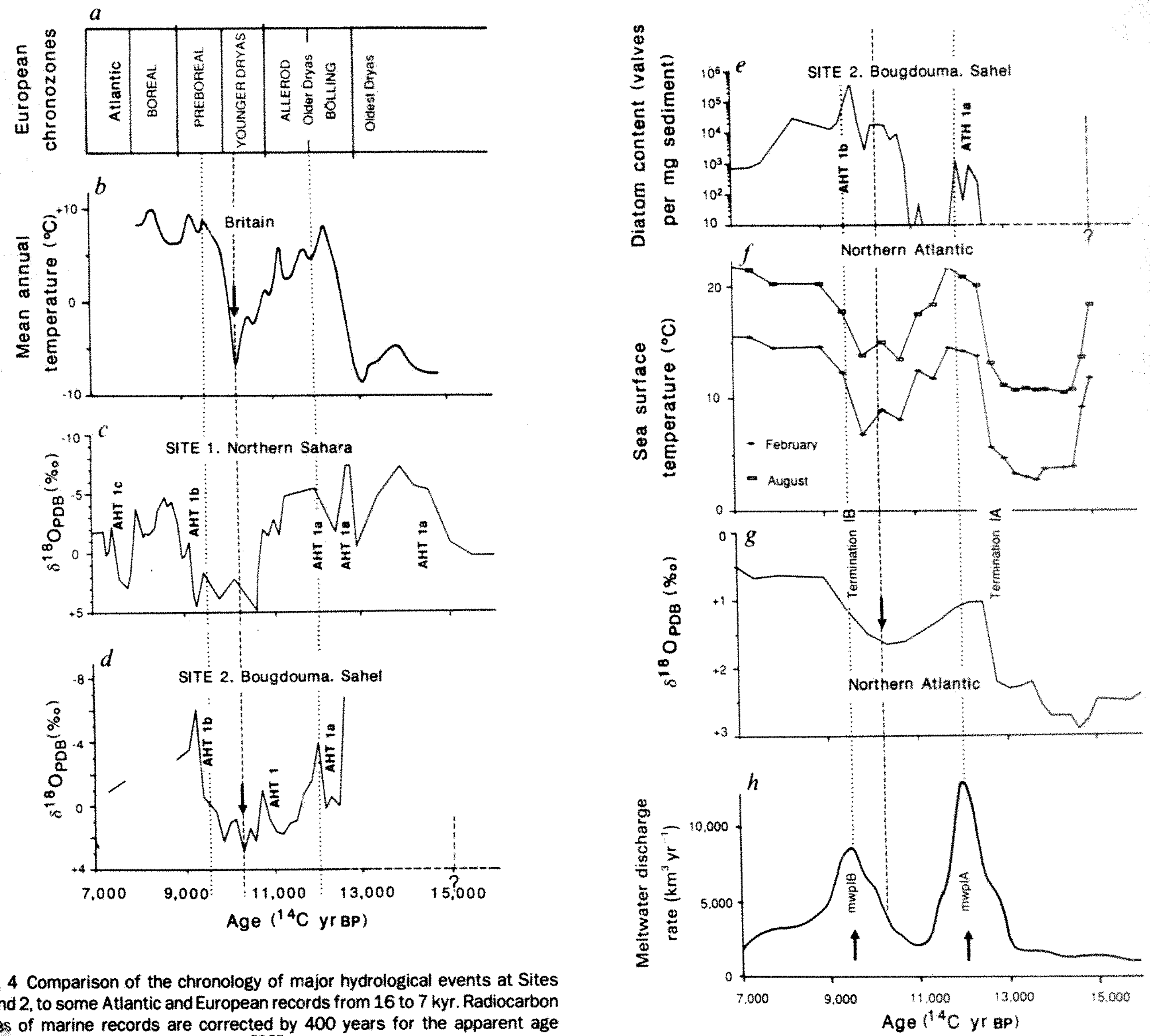

FIG. 4 Comparison of the chronology of major hydrological events at Sites 1 and 2, to some Atlantic and European records from 16 to 7 kyr. Radiocarbon ages of marine records are corrected by 400 years for the apparent age of sea water. *a*, European chronology[56,57]. *b*, The mean annual air temperature in Britain estimated from beetle remains[55]. *c*, Oxygen isotope profile of inorganic carbonate at Site 1, as an indicator of the ratio of groundwater influx to evaporation. *d*, Oxygen isotope profile of inorganic carbonate at Site 2, as an indicator of the ratio of groundwater influx to evaporation. *e*, Sediment content in the freshwater planktonic diatom *Cyclotella stelligera* var. *glomerata*, as an indicator of sudden rises of the aquifer[52]. *f*, The record of February and August sea surface temperature from the AMS ^{14}C-dated core SU81-18 located offshore of Portugal based on a palaeontological transfer function (ref. 4, and L. D. Labeyrie, personal communication). *g*, Oxygen isotope record of *G. bulloides* from core SU81-18 located offshore of Portugal[4]. *h*, The rate of the glacial melt water discharge calculated from Barbados sea level curve[6]. mwp = melt water pulse.

in sea surface temperature in the ocean core SU81-18[4], and the minimum mean air temperature in Britain[55] (Figs. 4*b*, *d*, *g*). In contrast with Site 1, however, sudden reversals are observed at Site 2, as in neighbouring sites[15,52], during the interval 12-10 kyr. A humid episode, from ~10.7 to 10.3 kyr, started in phase with the abrupt termination of the Younger Dryas recorded in Greenland ice cores[59]. Modern rainfall on the Sahel is statistically correlated with global SST anomaly patterns[60]. In the North Atlantic (core SU81-18) (ref. 4 and L. D. Labeyrie, personnel communication), the general drop in SST between 12 and 10 kyr is not monotonic (Fig. 4*f*). An increase of August SST observed from 10.7 to 10.3 kyr might be associated with the synchronous Sahelian humid phase.

Discussion

Major climate changes in North Africa during the last deglaciation seem to be in good chronological agreement with events deduced from deep-sea sediments, ice cores and European climatology. This indicates that the North African climate is largely subject to global (oceanic) control. The effects of cold Laurentide Ice Sheet melt water on the North Atlantic dynamics[8,20,21] have been invoked to explain the reduction in monsoon rainfall on the Sahel during the Younger Dryas[19]. But this does not account for the hydrological evolution at Site 1 because it is supplied by precipitation on the M'zab and on the Saharan Atlas Mountains, and is therefore difficult to attribute to a monsoonal climate. Nor does it explain the brief, but well defined, humid phase from ~10.7 to 10.3 kyr at 13-15° N, which shows that the concept of a unique recession synchronous with the Younger Dryas cannot be applied to the tropical latitudes of the Sahel. Other minor factors and feedbacks have to be investigated to explain the abrupt climate changes in this region between 11 and 10 kyr. Furthermore, the apparently variable timing of these events calls into question the notion that the Younger Dryas was a global event.

The principal factor inducing rainfall changes over Saharan and sub-Saharan Africa seems to have been the evaporative power of the atmosphere over the major production zones of air moisture. The atmospheric vapour made available by the global warming was distributed over Africa in response to several mechanisms: monsoon, squall lines, westerlies and Mediterranean depressions. We are now investigating the efficiency of air conveyors capable of carrying the vapour masses onto the African continent, especially in the central Sahara where the relative contributions of these effects may have varied during the year. Detailed surveys of laminated sediments are necessary to refine the seasonal variations for coupled atmosphere-ocean general circulation models. Such investigations are expected to reveal whether the different latitude belts of the Sahara and the Sahel could have been influenced by several distinct rainfall seasons during each year in the humid periods recorded by lacustrine events. □

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Evidence for interaction of different eukaryotic transcriptional activators with distinct cellular targets

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The adenovirus E1a protein (E1a), a potent transcription activator, contains a transcriptional activating region. Compared with previously described cellular and viral activators, E1a's activating region has unusual structural properties. It seems that E1a's activating region interacts with a cellular target not required for the function of transcriptional activators with 'acidic' activating regions. By contrast, the target of an acidic activating region is required both by acidic activators and by E1a. It is proposed that the cellular target of E1a's activating region is an 'adaptor' that allows E1a to interact with the basic transcriptional machinery.

THE 289-amino-acid E1a protein is required for the efficient expression of early viral genes[1-3]. Recent experiments provide evidence that E1a functions at the promoter and has the two essential properties of typical cellular activators: amino acids 121-223 contain both a promoter-binding region that directs E1a to its target promoters and a transcription activating region[4].

The transcription activating region of E1a[4] fused to the DNA-binding domain of GAL4 (amino acids 1-147)[5] increases transcription 200-fold from a core promoter bearing GAL4 binding sites. In contrast, this GAL4-E1a fusion protein does not activate transcription from a core promoter lacking GAL4 sites. The activating region revealed by these experiments is also required by the full-length E1a protein to activate an early viral promoter. Mutations that disrupt E1a's activating region can be rescued by the addition of a heterologous acidic activating region. Thus, E1a's activating region satisfies all the criteria used to define the activating regions of typical cellular activators.

Activating regions of several transcriptional activators have been mapped and grouped loosely into classes on the basis of their amino acid compositions. Activating regions defined to date are rich in acidic residues (GAL4, GCN4, VP16), glutamines (Sp1) or prolines (CTF/NF-I) (see ref. 6 for a review).

The acidic activating regions have been characterized most extensively. Studies of intact GAL4 and GCN4 show that the strength of an acidic activating region decreases only gradually upon deletion[7-9]. Analysis of the weak activating region of GAL4, region I, and activating regions generated at random from fragments of *Escherichia coli* DNA have verified the importance of acidic residues[10-13] but, in general, single amino-acid substitutions have little effect. These results suggest that activating regions are not highly structured, although some elements of structure are important for activity[10,11,14].

Although different acidic activating regions share no common sequence, they can substitute for one another and probably have a common cellular target[14]. Overexpression of an acidic activating region, for example, can inhibit the function of another acidic activator[11,15,16]. This inhibition, termed 'squelching', is thought to result from titration of the cellular target of the activating region. Accordingly, stronger activators inhibit (squelch) more effectively than weaker ones[11]. Overexpression of an intact activator can also have an inhibitory effect as a result of 'self-squelching'[14].

Here we present the results of mutational studies of E1a's activating region, revealing its unusual structural features. We also present squelching experiments indicating that the activating region of E1a interacts with a cellular target different from that of acidic activating regions.

Activating region of E1a

Boundaries. Our previous study showed that sequences of E1a between amino acids 139-223 comprise an activation region[4]. To define the boundaries of this region, we constructed derivatives of the previously described GAL4-E1a fusion protein which consists of amino acids 121-223 of E1a[4]. Figure 1 shows that the N-terminus of the activating region lies between amino acids 140 and 142: progressive N-terminal deletion to amino acid 140 resulted in a modest reduction in activity relative to intact GAL4-E1a. A drastic decrease in activity was observed on further deletion to amino acid 143. Activity was completely eliminated by deletion to amino acid 147. Site-directed mutagenesis of the two acidic residues at positions 140 and 141 shows that these positions are not critical for activation by E1a (see below). Thus, the N-terminal boundary of the activating region of E1a is located between amino acids 141 and 142.

Figure 1 also shows that the C-terminus of the activating region lies between amino acids 178 and 173: progressive C-terminal deletion to amino acid 178 did not decrease activity, whereas further deletion to amino acid 172 completely eliminated activity. We conclude that the core of the activating region has discrete boundaries and lies within E1a amino acids 141 and 178.

Sequences required for activation. The boundaries of the activating region indicate that it is contained within E1a region 3, a stretch of amino acids highly conserved among different adenoviruses[17] (Fig. 2*a*). To confirm the importance of these conserved amino acids for the function of the activating region, we constructed GAL4-E1a derivatives that contain single or multiple amino-acid substitutions. Figure 2*b* shows that with one exception (substitution of an arginine for the aspartic acid at position 168) mutation of one or more of the conserved amino acids disrupted the function of the activating region. With one exception (substitution of a cysteine for the tyrosine at position 159), mutations of the non-conserved amino acids had no effect.

Amino acids of all classes are conserved (Fig. 2*a*). Of the conserved amino acids, three are acidic, three are basic, and eighteen are uncharged. Mutational analysis (Fig. 2*b*) indicated that acidic, basic and neutral amino acids all contribute to form the E1a activating region. For example, basic amino acids at positions 160, 161 and 177, and neutral amino acids at positions 151, 154, 157, 165, 169, 170, 171 and 174 are all important for activity.

It was of particular interest to determine whether acidity is a determinant of the activating region of E1a. Substitution of acidic residues at positions 140 and 141 had little or no effect on activity. Changing the aspartic acid at position 168 to a basic residue did not substantially reduce activation. Substitution of a glycine for a conserved glutamic acid residue at position 148 (conserved in five of the six adenoviruses) reduced activity fivefold, whereas substitution of an alanine for the invariant aspartic acid at position 145 (conserved in all six adenoviruses) decreased activation fortyfold. Substitution of a glutamic acid for this aspartic acid reduced activity threefold, indicating that the role of this amino acid is not to provide negative charge *per se*. In summary, although at least one acidic residue (aspartic acid 145) is essential, net negative charge is not a determinant of E1a's activating region.

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Four of the conserved amino acids within the activating region are cysteines. These cysteines are the ligands of a metal binding site ('Zn finger') that is required for function of the E1a protein[18]. We find that these cysteines are essential for the function of E1a's activating region: changing one, two, or all four cysteines to alanines eliminated activity (Fig. 2*b*). As model studies have shown that the cysteine residues of Zn fingers are required for metal binding[19,20], we conclude that the binding of a metal ion is essential to the formation of the activating region.

Representative activating region mutants were tested for the stability of their protein products; each was expressed comparably to GAL4–E1a (Fig. 2*c* and data not shown). The small variations observed (less than threefold) cannot account for the large differences in activation. From the sum of these results we conclude that the E1a activating region is not acidic, is highly structured, and includes an essential metal-binding site.

Cellular target of the activating region of E1a

Inhibition by wild-type E1a. The significant structural differences between the activating region of E1a and those of other proteins, suggested that E1a might interact with a unique cellular target. To investigate the nature of this target we performed squelching experiments similar to those that have been carried out with other activators[11,14,21].

GAL4–E1a can efficiently activate the GAL4–E1b TATA reporter plasmid, whereas wild-type E1a cannot[4]. Presumably, this is because wild-type E1a is not efficiently directed to this synthetic promoter. We could therefore use wild-type E1a to compete with GAL4 derivatives for the target of its activating region. High levels of E1a inhibited transcription directed by the GAL4–E1a activator (Fig. 3*a*). In contrast, activation by strong (GAL4–VP16 and GAL4–regII) or weak (GAL4–regI) acidic activators was either unaffected or not significantly inhibited by coexpression of E1a (Fig. 3*a*). These results suggest that the activating region of wild-type E1a protein competes for the target of the identical activating region carried by GAL4–E1a. They further suggest that E1a's target is not required by the acidic activators, regions I and II of GAL4 or VP16.

Immunoprecipitation and immunoblot experiments indicate that the various GAL4 derivatives (GAL4–E1a, GAL4–VP16, GAL4–regII and GAL4–regI), which contain the same 147 N-terminal amino acids, were expressed at comparable levels (data not shown; see refs 4, 15). Moreover, when the amount of transfected DNA encoding the activator was reduced nearly 1,000-fold, the same specificity of inhibition was observed (see Fig. 6). Thus, the specificity of inhibition cannot be accounted for by variations in the levels of the different activators.

A prediction of these results is that GAL4–E1a would not be able to activate transcription in cells that express high levels of wild-type E1a. Figure 3*b* shows that in 293 cells, GAL4–VP16 but not GAL4–E1a can activate transcription from the GAL4–E1b–TATA reporter.

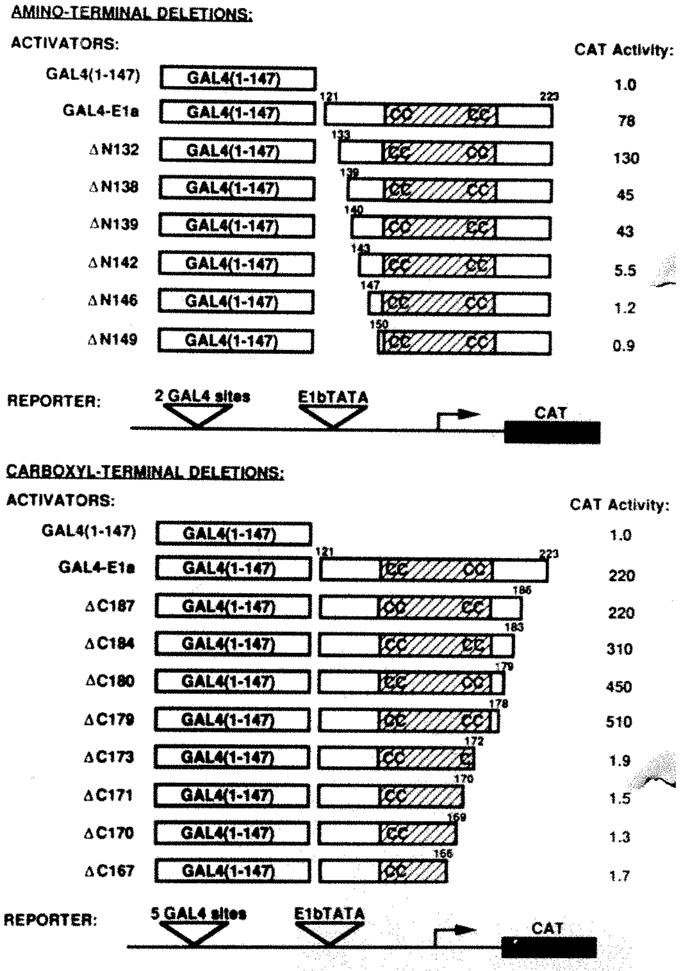
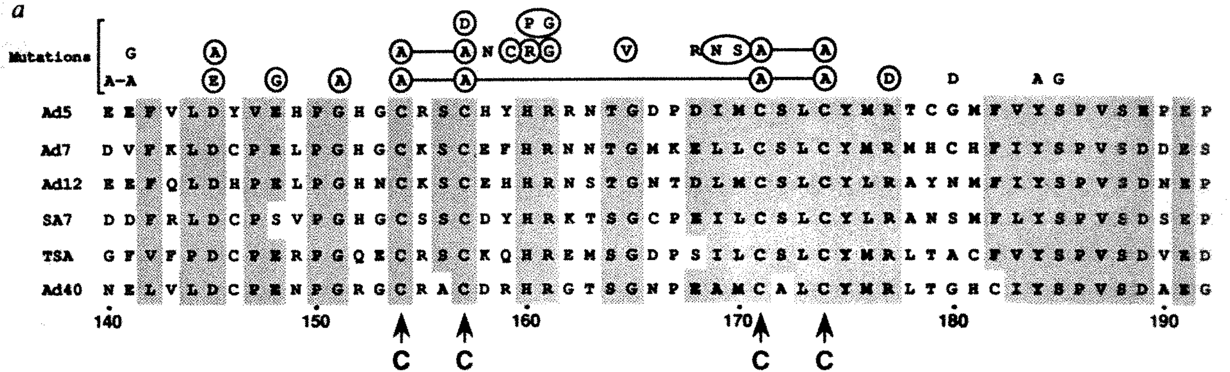


FIG. 1 The E1a transcriptional activating region lies between amino acids 140 and 178. A reporter CAT plasmid containing a core promoter (E1b TATA box) and two or five GAL4 binding sites was cotransfected with a plasmid encoding an activator protein. Relative CAT activity was measured and is indicated next to the diagrammed activators. The reporter plasmids are diagrammed below the sets of activators.

METHODS. Each GAL4 binding site is the 17-base pair (bp) oligonucleotide (MH100) previously described[33] and is inserted immediately upstream of each target promoter as indicated. The E1b TATA promoter contains an oligonucleotide comprising the E1b TATA box (5'-AGGGTATATAATG-3') inserted immediately upstream of the *CAT* gene in pSP72. GAL4 (1–147) is expressed from plasmid pSG147 or pSG424 (ref. 15). GAL4–E1a (pSGE1a) is E1a amino acids 121–223 fused in frame to the C-terminus of GAL4 (1–147), and was constructed by linking the *Cla*I–*Xba*I fragment of an adenovirus 5(Ad5) E1a 13S cDNA plasmid[34] downstream of GAL4 (1–147). GAL4–E1aΔN132, GAL4–E1aΔN138, GAL4–E1aΔN139, GAL4–E1aΔN142 and GAL4–E1aΔN146 are progressive 5' *Bal*31 deletions of the 13S cDNA *Cla*I–*Xba*I fragment, fused in frame to GAL4 (1–147). The GAL4–E1aΔC deletions are progressive 3' *Bal*31 deletions of the 13S cDNA *Cla*I–*Xba*I fragment, fused in frame to GAL4 (1–147). Unless otherwise stated, 2 μg per plate each of the reporter and activator plasmids were transfected by the DEAE dextran technique[35] into chinese hamster ovary (CHO-DUKX) cells grown on plates with a diameter of 10 cm. Cells were maintained below 50% confluency in α-MEM plus nucleotides and were split 1:8 24 h before transfection. Unless otherwise stated, 48 h post-dimethylsulphoxide shock cells were collected and assayed for CAT activity as described[36]. After autoradiography of the separated acetylated chloramphenicol forms, spots were excised and quantitiated by liquid scintillation counting. All assays presented have been repeated at least three times with comparable results.

Sequences required for inhibition. If the inhibition is due to squelching, then the sequences required for inhibition should be identical to those required for activation. To delineate the E1a sequences required for inhibition, we used as inhibitors the GAL4–E1a derivatives described above and assayed expression levels (Fig. 2*c*). We used a reporter chloramphenicol acetyl transferase (CAT) plasmid that contained six LexA sites upstream of the E1b–TATA box and was therefore activated by LexA–E1a but not by GAL4–E1a. The LexA–E1a activator contains the DNA-binding domain (amino acids 1–202) of the *E. coli* LexA protein fused to E1a (amino acids 121–223) (Fig. 3*c*).

Mutations within the E1a activating region, including the metal-binding site, reduced the ability of GAL4–E1a to inhibit LexA–E1a function. In contrast, mutations outside the E1a activating region did not affect inhibition by GAL4–E1a. Significantly, the degree to which each mutated activating region inhibited activation correlated with the strength of the mutated activating region. The cysteine mutations, for example, which completely eliminated activation (Fig. 2*b*), also completely eliminated inhibition (Fig. 3*c*). The E1a mutation 145A, which caused an intermediate level of activation (Fig. 2*b*), also caused an intermediate level of inhibition (Fig. 3*c*). We have tested all mutations in Fig. 3*a* that decrease activation and the rank order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A G
Ad5 E E F V L D Y V E H P G H G C R S C H Y H R R N T G D P D I M C S L C Y M R T C G M F V Y S P V S E P E P
Ad7 D V F K L D C P E L P G H G C K S C E F H R N N T G M K E L L C S L C Y M R M H C H F I Y S P V S D D E S
Ad12 E E F Q L D H P E L P G H N C K S C E H H R N S T G N T D L M C S L C Y L R A Y N M F I Y S P V S D N E P
SA7 D D F R L D C P S V P G H G C S S C D Y H R K T S G C P E I L C S L C Y L R A N S M F L Y S P V S D S E P
TSA G F V F P D C P E R P G Q E C R S C K Q H R E M S G D P S I L C S L C Y M R L T A C F V Y S P V S D V E D
Ad40 N E L V L D C P E N P G R G C R A C D R H R G T S G N P E A M C A L C Y M R L T G H C I Y S P V S D A E G
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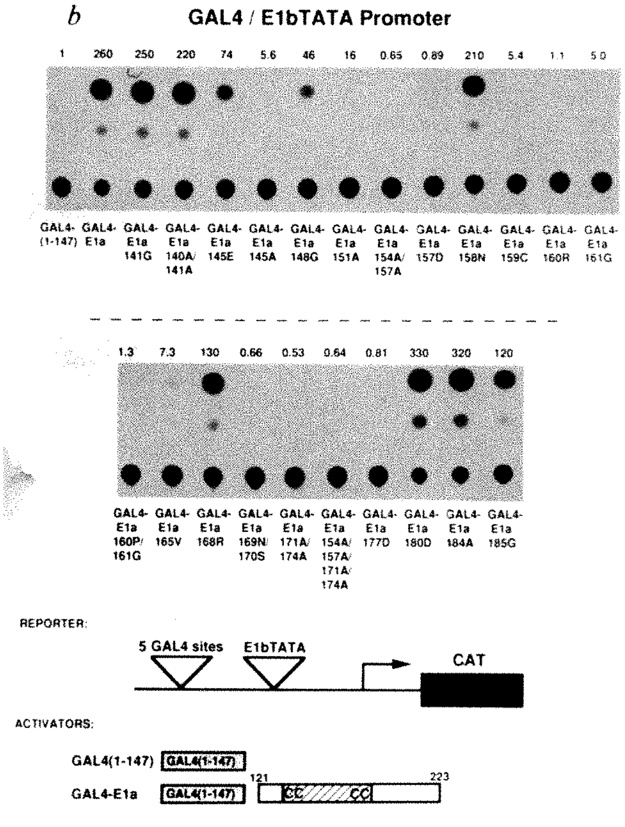


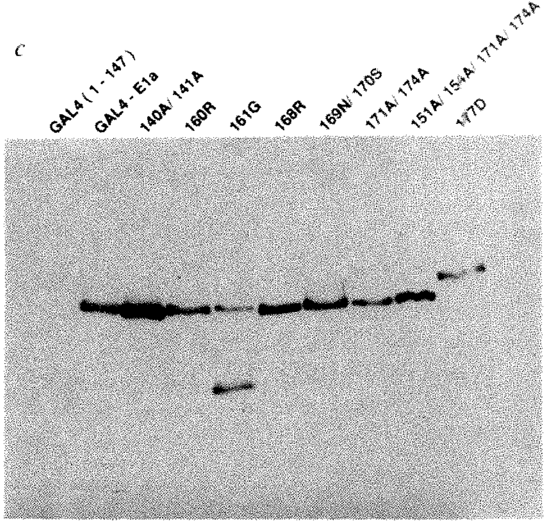
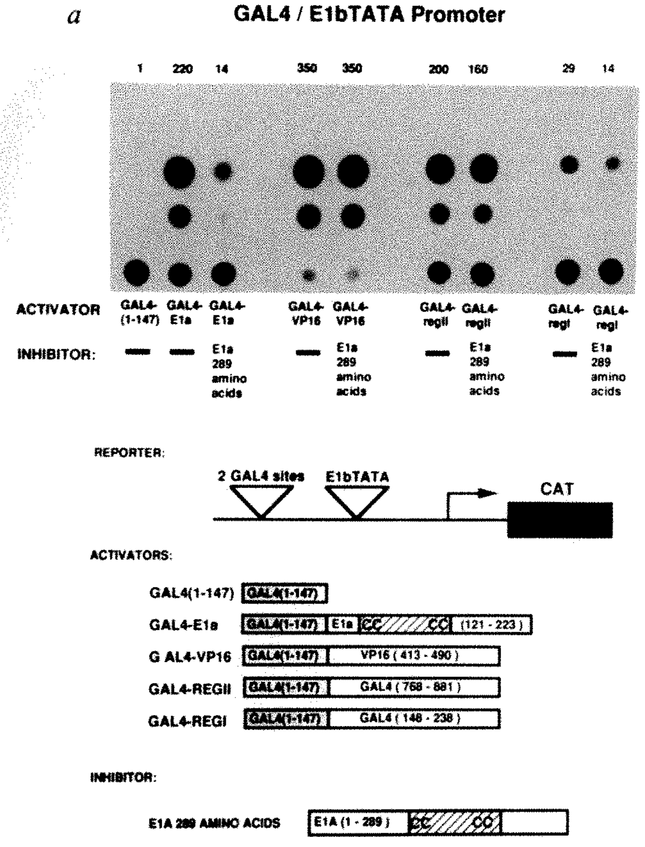


FIG. 2 Effects of amino-acid substitutions within Ad5 region 3. *a*, Region 3 amino-acid sequences (single-letter code) of six different adenoviruses[37,38]. The shaded regions represent those amino acids that are either strictly conserved in at least five of the six adenoviruses, or that have undergone conservative changes. The numbers below the sequences refer to the amino-acid position of Ad5. The bold Cs beneath the sequences indicate the positions of the four cysteines that chelate Zn^{2+}. The letters above the adenovirus sequences represent single, double and quadruple amino acid hanges we have constructed in Ad5 E1a region 3. Letters located above he mutated amino acid represent the residues that have been substituted. nes connecting letters indicate that multiple amino-acid substitutions have een made within the same molecule; circles that include more than one tter indicate that all enclosed substitutions were made in the same molecule. Letters not connected by lines or circled together represent single-residue substitutions. Those substitutions that had a greater than threefold effect on the ability of GAL4–E1a to activate transcription from a promoter containing GAL4 sites are circled. *b*, Transcriptional activity of mutant GAL4–E1a proteins. A reporter CAT plasmid containing a core promoter (E1b TATA box) and five GAL4 binding sites was cotransfected with a plasmid encoding an activator protein. Relative CAT activity was measured and is indicated above each lane of the autoradiogram. *c*, Expression levels of mutant GAL4–E1a proteins. ^{35}S-labelled GAL4–E1a derivatives from transfected COS cells were immunoprecipitated with an anti-GAL4 (1–147) antibody and fractionated on a polyacrylamide gel.

METHODS. *b*, GAL4–E1a180D contains a substitution at E1a amino acid 180 (G → D) and was constructed by replacing the *ClaI-XbaI* fragment of GAL4–E1a with the *ClaI-XbaI* fragment of the E1a activation mutant pH3G-13S-1098. All other mutations within region 3 were generated by site directed mutagenesis with mismatching oligonucleotides[39] or by random chemical mutagenesis[40]. *c*, COS cells were used to quantitate protein levels since higher levels of proteins are produced, facilitating detection. COS cells were transfected as described in the legend to Fig. 1. At 72 h post-transfection, cells were incubated with 200 μCi ml^{-1} of [^{35}S]methionine for 3 h. Labelled GAL4–E1a derivatives were immunoprecipitated[4] with an α-GAL4 (1–147) antibody (a gift of I. Sadowski) and fractionated on a 10% polyacrylamide gel. Unlabelled whole cell extracts were also prepared from COS cells cotransfected with plasmids encoding each activator and the GAL4–E1b-TATA–CAT reporter. These were assayed for CAT activity and results were identical to those obtained in CHO cells (data not shown).

in which they affected inhibition was the same as the rank order in which they affected activation (Fig. 2*b*, 3*c*, and data not shown). We conclude that the same E1a sequences are required for activation and inhibition. (This inhibitory effect is unrelated to the previously described E1a-mediated repression of enhancer-dependent transcription, which does not require conserved region 3 sequences[22-24].)

Figure 4 presents a control experiment which shows that coexpression of wild-type E1a with an E1a fusion protein (in this case LexA–E1a) did not alter the amount of fusion protein capable of binding to DNA. This assay made use of a reporter with a LexA site located between the TATA box of the SV40 promoter and a *CAT* gene. Proteins that bind to this LexA site inhibit transcription[35]. Under the identical conditions that resulted in a large inhibition by the wild-type E1a of LexA–E1a activation (Fig. 4, left panel), there was no change in the level of DNA binding by LexA–E1a (Fig. 4, right panel). At the level of LexA–E1a that was used (0.5 µg DNA), this binding assay was sensitive to changes in protein levels (Fig. 5, bottom panel of graph). Neither LexA–E1a nor wild-type E1a affected transcription from an SV40-*CAT* reporter lacking LexA sites, and wild-type E1a did not affect transcription from the SV40-LexA-*CAT* reporter (data not shown).



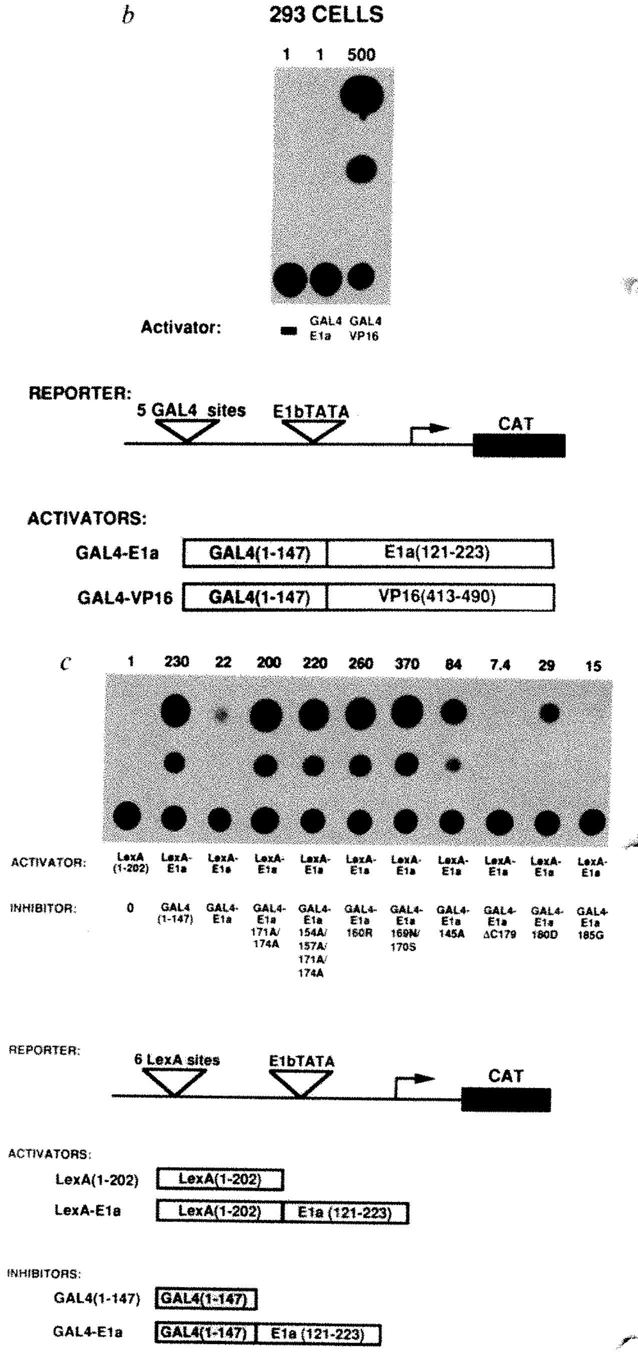


FIG. 3 Inhibition of transcription by E1a. *a*, Wild-type E1a. Reporter CAT plasmid (2 µg) containing a core promoter (E1b TATA box) and two GAL4 binding sites was cotransfected with 2 µg of a plasmid encoding a GAL4 derivative and, where indicated, 2 µg of either an E1a expression plasmid or the pSP64 (Promega) vector. Relative CAT activity is indicated above each lane. The structures of the target promoters and activator proteins are diagrammed below. *b*, 293 cells. The reporter diagrammed (1 µg) and 2 µg of the specified activator were transfected into 293 cells by the DEAE dextran technique[35]. In the absence of activator, 2 µg of vector was transfected. Cells were collected 72 h post-dimethylsulphoxide shock. *c*, GAL4–E1a and E1a mutants. Reporter CAT plasmid (1 µg) containing a core promoter (E1b TATA box) and six LexA binding sites was cotransfected with 40 ng of a plasmid encoding an activator protein and, where indicated, 1 µg of plasmid encoding an inhibitor protein. In the absence of inhibitor, 1 µg of vector was added. Cells were collected 72 h after DMSO shock.

METHODS. GAL4–regI is the weak GAL4 activating region fused to GAL4 (1–147) and is expressed from pSG238 (refs 5, 7). GAL4–regII is the strong GAL4 activating region fused to GAL4 (1–147) and is expressed from pSG236 (refs 5, 7). GAL4–VP16 contains the 78 C-terminal amino acids of the HSV1 VP16 protein fused in frame to the C-terminus of GAL4 (1–147)[15]. Wild-type E1a is the 289-amino-acid E1a protein expressed from a plasmid (pSP613S) that contains the E1a region of adenovirus type 2 (minus the 13S intron) inserted into pSP64 (Promega). The LexA–E1bTATA reporter (pL6EC; a gift from P. Broad) has a triplicated insert of a 41-bp *Xho*I fragment, which contains two LexA sites derived from the *colE1* promoter[41] in the *Xho*I site of the E1b TATA reporter. LexA (1–202) comprises LexA amino acids 1–202 and 12 amino acids coded for by a polylinker and is expressed from the plasmid pBXL1 (gift from P. Broad). This plasmid is analogous to pSG424 (ref. 42), but contains LexA (1–202) in place of GAL4 (1–147). LexA–E1a is E1a amino acids 121–223 fused in frame to the C-terminus of LexA (1–202) and was constructed by inserting the *Eco*RI-*Xba*I fragment of pSGE1a (GAL4–E1a) downstream of LexA (1–202), between the *Eco*RI and *Xba*I site of pBXL1.

Self-squelching of E1a derivatives. We next determined the effect of overexpressing a single E1a derivative. Figure 5 shows that increasing the amount of transfected DNA expressing either GAL4-E1a or LexA-E1a led at first to increased activation and subsequently to inhibition. The bottom panel of the graph confirms that the level of LexA-E1a protein correlated with the amount of transfected DNA. The intracellular level of LexA-E1a protein increased steadily over the course of the titration as assayed by its ability to bind *in vivo* to a LexA site located between the SV40 promoter and a *CAT* reporter gene[25]. Proteins that bind to this LexA site inhibit CAT expression. Expression from an SV40-*CAT* reporter that lacks LexA sites was unaffected by cotransfection of the plasmid expressing LexA-E1a (data not shown).

These results suggest that, as the intracellular concentration of the E1a derivative is raised, activation of transcription increases until all DNA sites or all activation targets are saturated. After that, overexpression of the E1a derivative reduces the level of activation as the unbound E1a derivative begins to sequester the activating target. Self-squelching by LexA-E1a and GAL4-E1a show that E1a squelching is not a consequence of the coexpression of two different E1a derivatives (for example, by formation of inactive heterodimers).

Cellular target of the activating region of VP16

Expression of the E1a activating region can inhibit transcription directed by GAL4-E1a or LexA-E1a but not by the acidic activating regions of GAL4-VP16, GAL4-regI or GAL4-regII (Fig. 3). This indicates that the target of the E1a activating region is not required for transcription directed by acidic activators. To address whether acidic activators use targets that are required for transcription directed by E1a, we asked whether VP16 can squelch E1a.

Figure 6 shows that, as expected, VP16 can compete for its own activation target: LexA-VP16 inhibited transcription activation by GAL4-VP16. The target of VP16's activating region is also required for activation by E1a: LexA-VP16 inhibited activation by GAL4-E1a. As with the full-length E1a protein, an E1a fusion protein competed for its own activation target (LexA-E1a inhibited GAL4-E1a), but did not inhibit transcription directed by the VP16 activating region. We are not merely observing an ability of the inhibitor to squelch any activator of equal or weaker strength than itself, as neither E1a nor LexA-E1a can squelch the relatively weak GAL4-regI, or GAL4-regII (Fig. 3*a* and data not shown). Thus, we conclude that E1a's activation target is not required by VP16, whereas VP16's activation target is required by E1a.

Discussion

Activating region of E1a. The activating region of E1a has relatively discrete boundaries and is inactivated by a number of single amino-acid substitutions. These essential amino acids are of several classes. Taken together, our mutagenesis experiments suggest that the E1a activating region is highly structured and that this structure is important for activity. It is likely that the metal-binding site, which is essential for function, has an important structural role. Metal-binding sites can allow proteins to form highly structured and essentially independent domains from a limited number of amino acids[20].

Many sequence-specific DNA-binding proteins contain metal-binding sites, termed Zn fingers[19,20,26]. It is not clear whether E1a's metal-binding site is involved in DNA binding. Although E1a is not a sequence-specific DNA-binding protein,

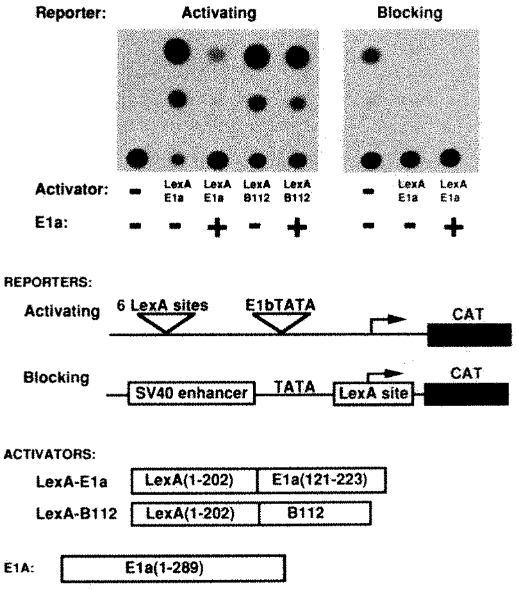


FIG. 4 *In vivo* DNA-binding assay. The diagrammed reporter (1 μg), 0.5 μg of the specified activator or vector DNA, and 1 μg of E1a expression plasmid or vector DNA were transfected into CHO cells. Cells were collected 56 h post-DMSO shock. The two panels represent transfections done in parallel with identical procedures, only the reporter DNAs differ. LexA-B112 (a gift from P. Broad) is LexA (1-202) fused at its C terminus to B112, which is an acidic amphipathic amino-acid sequence derived from random *E. coli* DNA that functions as an activator of transcription[10].

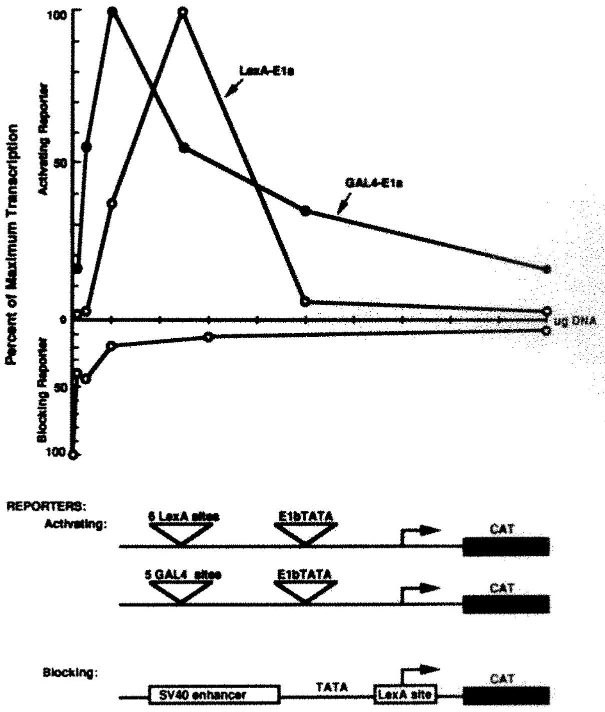


FIG. 5 High levels of either GAL4-E1a or LexA-E1a inhibits transcription activation. Activating reporter CAT plasmids (1 μg) containing a core promoter (E1b TATA box) and either six LexA sites or five GAL4 sites were cotransfected with varying amounts of plasmids encoding the appropriate E1a fusion protein, LexA-E1a (○) or GAL4-E1a (●). The total amount of DNA transfected was held constant by including an appropriate amount of vector DNA. Each mark on the x-axis represents 1 μg of activator DNA; a minimum of 100 ng and a maximum of 10 μg of activator DNA were transfected per 10 cm plate of cells. Cells were collected 72 h post-dimethylsulphoxide shock. Relative CAT activity was measured and is graphed as a percentage of the maximum level of CAT activity for each DNA titration. The data plotted below the x-axis are CAT expression levels from a cotransfection of the blocking reporter diagrammed, also called VV(X)CO[25], and varying amounts of DNA encoding LexA-E1a. Relative CAT expression levels are reported as a percentage of the level of CAT expression observed in the absence of LexA-E1a. The lower plot is an indication of the intracellular concentration of the LexA-E1a protein. Structures of the target promoters are diagrammed below.

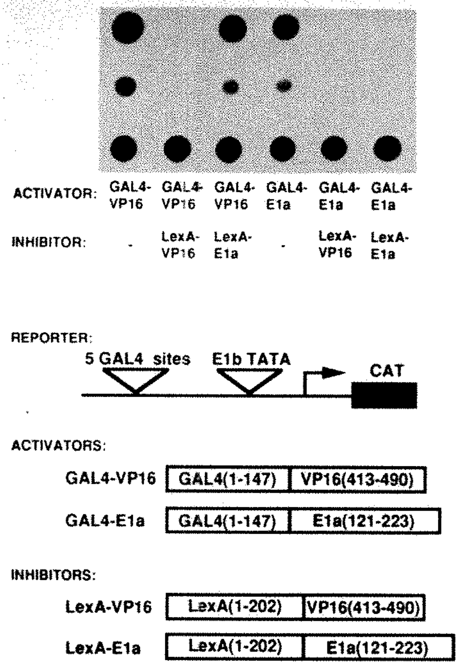


FIG. 6 Inhibition by VP16 and E1a transcriptional activating regions. A reporter CAT plasmid (1 μg) containing a core promoter (E1b TATA box) and five GAL4 sites was cotransfected with plasmids encoding an activator and, where indicated, an inhibitor protein. 3 ng of the GAL4-VP16 activator and 10 ng of the GAL4-E1a activator were used. The inhibitors were both used at a level of 3 μg.

METHODS. LexA-VP16 (gift from P. Broad) and contains the 78 C-terminal amino acids of HSVI VP16 protein fused in frame to the C-terminus of LexA (1-202) in the expression vector pBXL1. Cells were collected 72 h post-DMSO shock. CAT assays of the GAL4-E1a activators (lanes 4-6) were performed with 25-fold more cell extract than those of the GAL4-VP16 activator (lanes 1-3).

it may bind to DNA nonspecifically[27]. The E1a region responsible for DNA binding and its relevance to transcription remains to be determined. Berg[20] has pointed out that E1a's metal-binding site bears structural similarities to the metal-binding site of aspartate transcarbamylase, which is not a DNA-binding protein. The metal-binding site of aspartate transcarbamylase is at a surface involved in protein-protein interaction[28]. We favour the idea that the principal role of E1a's metal-binding site is to form a surface that interacts with other proteins.

Adaptors. We suggest that the inhibition observed at high concentrations of the E1a activating region is caused by sequestration of its cellular target. Our interpretation is like those reached in studies of other activating regions[11,14-16]. We recognize that neither our nor previous results prove the existence of a limiting cellular target, or that competition for it caused the inhibition. We cannot, however, think of an alternative interpretation that adequately explains these results. Moreover, our demonstration that there is specificity to squelching significantly strengthens this interpretation as it rules out the possibility that the inhibition is caused by cytotoxicity or a nonspecific inhibitory effect on transcription, for example.

We refer to E1a's target, inferred from these experiments, as an 'adaptor' because it is not required for transcription directed by acidic activators. E1a's target is therefore an auxiliary, rather than an essential, transcription component. In contrast, the target of acidic activating regions is required for transcription directed both by VP16 and by E1a. This could be explained if acidic activating regions directly interact with an essential transcription component. In fact, DNase I footprinting studies provide evidence for an interaction between an acidic activating region and the TATA-box binding factor, TFIID[29].

We imagine that E1a's adaptor binds to E1a and also interacts directly with the basic transcription machinery. For example, the adaptor may contain, in addition to an E1a binding surface, an acidic activating region. A precedent for such an adaptor is the viral VP16 protein, which contains a potent acidic activating region[15,16] and a second region that interacts with the cellular transcription factor Oct-1[30,31]. By itself, Oct-1 cannot activate transcription from a typical promoter containing a TATA box. The same promoter can, however, be activated by Oct-1 in the presence of VP16[32]. It is conceivable that other nonacidic activating regions, such as those rich in glutamines or prolines, may also act through specific adaptors. Such adaptors would explain how diverse protein motifs can all activate transcription through the same basic transcription components. □

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Opaque spiral galaxies

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SPIRAL-galaxy disks have long been thought to contain only a modest amount of light absorbing dust, meaning that they are transparent to their own starlight, and that observers see light from their far sides. This view was based on studies of the variation of the average surface brightness as a function of the galactic inclination along the line of sight in small samples of galaxies[1,2]. We[3] have digitized the photographic images of a carefully selected sample of about 16,000 galaxies and determined an extensive set of photometric parameters. These data allow a re-analysis of the effective transparency, using the measured surface brightness profiles for much larger, more strictly defined samples. Several tests provide evidence that the major parts of many spiral disks are opaque, and that in many cases perhaps only the outer layer of stars is observable. This invalidates most determinations of mass-to-light ratios. Various photometric properties point to an obscuring component with a larger exponential scale length than that of the stars, possibly composed of cool compact opaque clouds. This result diminishes the evidence for dark haloes around spirals, as inferred from rotation curves.

Classically, the extinction inside the disks of spiral galaxies is studied by selecting a group with supposedly similar intrinsic properties and analysing how various parameters change with the inclination relative to the line of sight, the inclination angle being derived from the observed axial ratio a/b. For a transparent disk the apparent total blue magnitude B is constant, whatever the inclination, but for an optically thick disk, B varies approximately as $B_{\rm obs} = B_{\rm face} + 2.5\,D \log(a/b)$, where $D \doteq 1$ for a fully opaque disk. The variation of the local surface brightness μ^B as a function of inclination is reversed. For the optically thick case, μ^B is constant with inclination because the observer will always see about one mean free path into the system. In the transparent case, the longer line of sight through inclined systems will brighten the apparent μ^B approximately as

$$\mu^B_{\rm obs} = \mu^B_{\rm face} - 2.5C \log(a/b) \qquad (1)$$

where $C = 1$ if there is no extinction at all.

Disney *et al.*[4] recently reviewed the weak evidence for transparency of spiral galaxies and pointed out that various standard models[1,2,5,6] correspond to values of C in the range 0.46–0.9 (semi-transparent to transparent). The models are based on Holmberg's original studies[1] of the relationship between the 'projected surface brightness' $\mu^B_{\rm proj}$ and axial ratio for a sample of 119 spiral galaxies. He measured the total magnitude within the $\mu^B = 26.5$ isophote together with the major and minor diameters a and b, and from these derived $\mu^B_{\rm proj} = B + 2.5 \log a^2$. Anticipating semi-transparent systems, he successfully fitted an 'absorbing screen' model to $\mu^B_{\rm proj}$ as a function of a/b. If disks are fully opaque, however, a is not expected to vary with inclination. This implies that the variation of $\mu^B_{\rm proj}$ with a/b can in fact represent the expected variation of B in the opaque situation. For axial ratios up to $a/b = 5$, Holmberg's original data points can be fitted quite well with an exclusive (opaque) $D = 1$ correction to B. With the thousands of data points from the ESO-LV survey (ref. 3), Holmberg's measurements of the regression of $\mu^B_{\rm proj}$ with a/b is confirmed, but this classical test, which for several decades has been thought to indicate that the disks of spiral galaxies are transparent, can be interpreted equally well using the optically thick model. The ambiguity arose because of the use of the average surface brightness to study a/b-dependent terms, but now measures of the local surface brightness are available.

Here I report tests using the diameter-complete sample of 9,381 late-type galaxies extracted from the ESO-LV survey, that is, a sample of galaxies for which full surface-brightness profiles have been measured. Three measures were used: the extrapolated central surface brightness $\mu_0^{B,\rm fit}$ found by fitting an exponential to the two-dimensional light distribution, the μ_0^B within a 1″ aperture converted from the measured surface brightness within an aperture of radius 5″, $\mu^B_{5''}$, and the observed surface brightness at the half-total-light radius μ_e^B. Table 1 lists the means of these three independently derived values of the central surface brightness for different morphological types. For pure exponential disk systems without a bulge, $\mu_0^B = \mu_e^B - 1.822$. Columns 4, 6 and 7 demonstrate the consistency between the three different techniques and column 5 shows the very small standard deviation of μ_0^B. A comparison of μ_0^B with the other two measurements shows that the presence of bulges in early-type spirals (types 1 and 2) increases the actual central flux by only ~0.3 m (m is relative magnitude); but results for these types could be affected by bulges. For the later types, no significant contribution from bulges is observed.

The key test, the analysis of the relationship between surface brightness and axial ratio, is presented in Fig. 1. This provides the strongest evidence that the disks of spiral galaxies are not transparent and, as the surface brightness at the half-total-light radius (diameter D_e) hardly brightens with a/b, this must be so for large parts of the disks. For different morphological types, equation (1) has been fitted to both μ_0^B and μ_e^B as function of $\log(a/b)$. The resulting coefficients, μ at $a/b = 1$ and C, are listed in Table 1. The central areas of all spirals of types 1–6 behave as would be expected for opaque conditions; this result was also found using the uncorrected $\mu^B_{5''}$. This condition seems to relax at the half-total-light radius of Sc and later types and at the centres of Sd galaxies. Beyond the half-total-light radius, any further radiation variation of C can be estimated using the distance-independent ratio D_{26}/D_e where D_{26} is the diameter

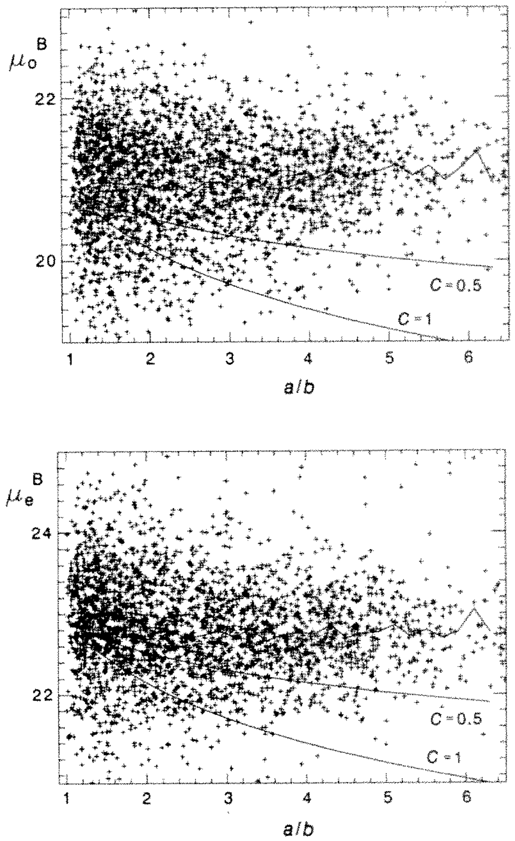

FIG. 1 The distribution of 2,639 type 2.5–5.0 (~Sb) galaxies in the surface brightness (left, at centre; right, at half-total-light radius) a/b diagram. The mean μ in a/b bins of 0.2 is indicated together with two curves representing the expected trend for transparent disks with $C = 1$ and $C = 0.5$.

TABLE 1 Comparison of different measures of central surface brightness of 9,381 late-type galaxies and deduced transparencies

	Type	Total number	μ_0^B mean	μ_0^B s.d.	$\mu_e^B - 1.8$ mean	$\mu_0^{B,\mathrm{fit}}$ mean	μ_0^B $a/b=1$	C_{μ_0} all	C_{μ_0} control	μ_e^B $a/b=1$	C_{μ_e} all	C_{μ_e} control	C_{1-D}	Number of controls
1	Sa	1,329	20.51	0.71	20.85	21.01	20.51	−0.01	−0.10	22.98	0.42	0.44	0.20	308
2	Sa-b	646	20.71	0.68	21.05	21.17	20.67	−0.06	−0.05	23.17	0.34	0.48	0.20	136
3	Sb	1,873	20.96	0.69	20.99	21.19	20.95	−0.02	−0.02	22.94	0.13	0.08	0.15	323
4	Sb-c	807	20.89	0.71	21.01	21.24	20.85	−0.08	−0.01	22.90	0.13	0.05	0.15	187
5		828	21.15	0.62	21.17	21.47	21.01	−0.18	−0.05	23.10	0.10	0.10	0.15	125
6	Sc	1,395	21.52	0.59	21.41	21.65	21.62	+0.10	+0.02	23.43	0.19	0.22	0.20	439
7		659	21.71	0.58	21.66	21.90	21.84	+0.14	+0.06	23.80	0.30	0.37	0.20	101
8	Sd	524	21.96	0.55	21.95	22.12	22.24	+0.32	+0.12	24.10	0.37	0.32	0.28	63
9		590	22.36	0.70	22.41	22.51	22.74*	+0.50*	+0.44	24.57*	0.57*,†	0.27	0.28	81
10	Irr	730	22.27	1.03	22.59	22.34	22.85*	+0.76*	+0.41	24.62*	0.79*,†	0.39	0.28	104
Dwarfs‡ 3–7		100	21.34	0.66	21.24	21.49	21.39	+0.18	-	23.16	0.24			

μ not corrected for galactic extinction. * Using only exponential-profile systems. † $C=0.3$ for whole sample. ‡ $M_B > -19$, Hubble constant $H_0 = 50$ km s^{-1} Mpc^{-}

at $\mu^B = 26$ isophote. The a/b dependency of this ratio is related to the variation in transparency between the two diameters. The ratio is roughly constant for equal transparency and the ratio increases with a/b for all situations of radially increasing C. For types 2–6, no significant variation of D_{26}/D_e with a/b is found, which implies that the C_{μ_e} values listed in Table 1 must also apply to the outer regions of these galaxies.

The formal statistical errors of the listed C values are very small (≤ 0.03) and the implications of the low values obtained are so crucial for understanding the contents of spiral disks that a detailed evaluation of the tests is necessary. First, the tests were repeated using only those objects for which a free fit indicated that they possess a perfect exponential light profile. This reproduced the C values within the uncertainties and makes it very unlikely that the result is affected by the presence of bulges, at least for types 3–10. Second, the position of D_e could depend on a/b. In general, however, for both transparent and opaque systems the definition of D_e precludes such a dependency. As a check, C values were determined using $\mu_0^{B,\mathrm{fit}}$, which resulted from a fit to the two-dimensional light distribution between $r = 5''$ and the 25th isophote. These values are insensitive to the actual location of D_e and reproduce those obtained from μ_e^B, within 0.03. Because for most systems D_e is located in the middle of the area used for the fit, this also confirms the small radial variation of C.

Finally, selection effects inherent in a diameter-selected sample[3] may have influenced the result. At fainter magnitudes the survey has a bias against bright μ_0 and low-a/b systems (Fig. 2). By reducing the total sample to 1,867 objects, with $\mu_B < 21$ for types 1–5 and $\mu_B < 22$ for types 6–10, these selection effects are avoided. This 'control' sample has a distribution of observed a/b ratios very close to that expected from a randomly acquired sample of intrinsically round disks. Moreover, it is most complete with respect to its selection criteria. As seen in Table 1, the control samples reproduce the C values from the total sample very well, also demonstrating that axial-ratio selection effects are not affecting the result.

Another way of visualizing the result, using the total sample, is by counting the number of objects that are positioned on the right side of the curve $\mu_{\mathrm{lim}} = 26$ in Fig. 2. An intrinsically round and face-on exponential disk galaxy will be a part of the sample if it lies to the left of this 'inclusion boundary'[3,7,8]. All 4,873 galaxies (52%) on the right side of this curve, if they were intrinsically round, passed that boundary because of inclination effects. As their a/b is known the surface brightness and/or the magnitudes of these galaxies can be adjusted to their face-on values. When a 'transparent' correction ($C=1$) is applied to μ_0^B, 36% remain on the right side, for a 'semi-transparent' correction ($C=0.5$, $D=0.5$) the fraction is 23%, whereas for the 'fully opaque' correction on B_T ($D=1$), it is only 9%. As the curve describes the prime selection effect of a diameter-selected sample, this demonstrates that the data fully match the selection effect for opaque spirals, whereas transparent systems would lead to a serious inconsistency.

The low C values for large radii also imply a rather modest dependency of isophotal diameters on a/b (contrary to ref. 5) This allows an independent check on the result, by analysing for which magnitude correction the scatter in the "absolute total magnitude intrinsic diameter" reduction is a minimum. For a subsample of 1,582 spirals a minimum scatter is found fo $D=0.85$. For simple models, $C=1-D$, and estimates of C_{1-D} calculated from D are listed in Table 1. Magnitudes that were not corrected for inclination ($D=0$, transparent) resulted in a r.m.s. scatter of 0.64, whereas for an opaque ($D=1$) correction the scatter was reduced to 0.47. This reduction is highly significant (8.5σ) and confirms that most parts of the disks are opaque.

The tabulated C values can be used as an overall diagnostic on the effective transparencies at different locations for different types and can be applied directly to estimate face-on magnitudes for inclined systems. If $C \leq 0.2$ for large parts of the disks, as found for Sb and Sc galaxies, very strong obscuration is implied, requiring a dramatic revision of the interpretation of the surface-brightness profiles. For the centres, detailed modelling indicates that negative C values can occur, due to extinction of the light originating from opaque layers by surrounding semi-transparent layers. The amazingly small variation of μ_0^B (refs 9, 10) is also confirmed (contrary to refs 11, 12) albeit with the refinement that the average μ_0^B becomes larger with increasing type, which for later types (7–10) is also correlated with larger M. For opaque

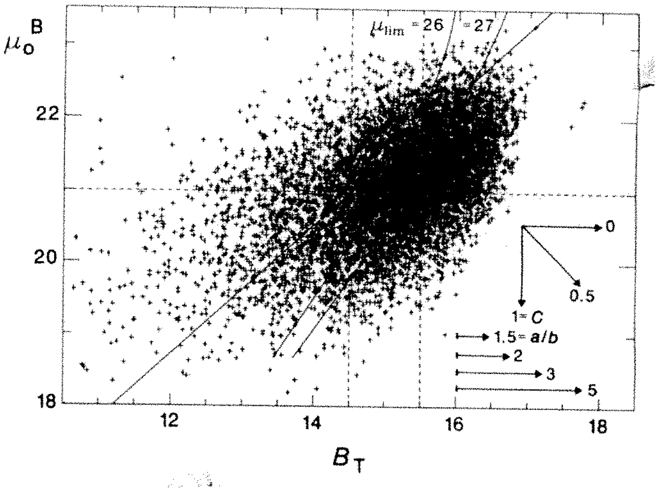

FIG. 2 A plot of central surface brightness against total magnitude for 8,061 spiral galaxies (type 0.5–8.5). Direct observational data, not corrected for inclination. The $\mu_{\mathrm{lim}} = 26$ curve represents the theoretical inclusion boundary for circular face-on exponential disks and corresponds to the 1′-diameter selection criterion of the survey. The vectors show how intrinsically circular disks move in the diagram for various transparencies (direction) and inclinations (amplitude). The positions of 4,873 galaxies to the right side of the $\mu_{\mathrm{lim}} = 26$ curve can only be understood if most of them represent inclined opaque disks. The straight line shows the observed regression for face-on circular disks. At fainter magnitudes the survey has a bias against bright μ_0 objects and low-a/b systems.

conditions, this small variation would result from a limited range of star-to-dust ratios for each morphological type, which on average should then also vary with type.

An observed value of $C = 0.2$ for large parts of Sb and Sc disks with a modest radial variation has even greater consequences. The observations cannot result from opaque layers with a small scale height compared to the stellar distribution, because the photometric properties of such disks would then be like those of transparent systems (μ_B decreasing with increasing a/b, contrary to the observations) and we would not even notice that we miss half of the starlight. A measurement of $C = 0.2$ at a particular radius could result from a two-layer situation in which 80% of the detected light originates from a fully opaque layer with optical depth $\tau \gg 1$ and the rest from a surrounding transparent layer. Calculations[13] also demonstrate that the effect of multiple scattering on dust grains can produce a C value around 0.2 in $\tau \gg 1$ disks. Models incorporating a $\tau \gg 1$ layer would predict constant surface brightness profiles, however, given the observed modest radial variation of C. This also applies to the models of ref. 4 where the absorption is thought to be due to cirrus-like distributed dust. Areas of equal surface brightness can indeed be found along the arms of various spirals and a flat profile is also seen in several central areas. But many exponential light profiles are observed in $C = 0.2$ disks. This implies that the detected light and its corresponding C should originate mostly from a single layer of well mixed stars and obscurers, with $\tau \approx 1.3$ corresponding to ~0.6 m total face-on extinction, in which case about half of the system would be detected when face-on and one-sixth when at 80° inclination. The radial exponential intensity distribution should then be related to the radial volume density of the stars (ρ_*), the volume density ρ_0, and cross-section σ_0 of the obscuring material by $I(r) \approx (\rho_*(r)/\rho_0(r)\sigma_0(r))(1-e^{-\tau})$. For a modest variation of τ this implies either a radially decreasing star-to-obscurers density ratio, or increasing σ_0, or both. In fact, the obscuring bodies must have the property $\tau = T\rho_0\sigma_0 = 1.3$, with T of the order of a hundred parsecs. This condition can be evaluated for all scales and the most reasonable match is with compact opaque clouds with, for instance, diameters of the order of 50 pc and $\rho_0 \approx 6\times10^{-6}\,\mathrm{pc}^{-3}$. These clouds should contain dust and stars that are in turn embedded in a sea of stars. As the distance to the galactic centres increases, the ratio of the volume density of the clouds to that of 'the sea of stars' should increase. The expected low temperatures for such clouds predict a substantial flux of submillimetre-wavelength radiation from Sb and Sc disks, which has indeed been observed[14].

Whatever the true nature of the light-absorbing bodies, if they carry mass the implication is that the mass distribution in the disks will have a larger exponential scale length than that indicated by the observed light profile. In other words, the observed mass-to-luminosity ratio will increase with radius, despite the fact that the result implies an increase in true luminosities. This indicates a mass component that can produce flat rotation curves without the introduction of haloes of dark matter[15]. Successful fits of the rotation curve, with only disk material and obeying the extinction properties as presented here, have been made[16]. This result seriously weakens the evidence for dark haloes in Sb and Sc galaxies. Neither does it require alternative theories of gravity[17] to explain the flat rotation curves. This result does not effect the evidence for dark haloes in elliptical galaxies[18,19] (rotation velocities of which are, however, very difficult to explain with modification to Newton's law) or the missing-mass problem in spiral-poor clusters of galaxies, like the Coma cluster. ☐

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Electronic properties of junctions between silicon and organic conducting polymers

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DIODES formed from semiconductor/metal interfaces often display non-ideal electronic properties. For instance, silicon/metal (Schottky) diodes made from n-type silicon and a variety of contacting metals exhibit only small differences in their rectification properties, despite theoretical and practical expectations that changes in the metal should effect changes in device properties[1,2]. Similarly, Schottky diodes formed on p-type silicon generally exhibit ohmic behaviour with poor rectification characteristics. This lack of electrical response to changes in the properties of the contacting metal phase is generally attributed to interfacial reactions that take place during the high-temperature thermal or electron-beam deposition of metals onto silicon[3]. Here we describe the fabrication of diodes using a low-temperature chemical procedure, in which contact to the semiconductor is made by a layer of the conducting organic polymer, polyacetylene. Unlike conventional metals, the electrical properties of polyacetylene can be manipulated through choice of the polymer dopant. The resultant organic/inorganic interfaces behave more ideally than contacts with conventional metals, in that changes in the electrical properties of the conducting polymer exert a large and predictable effect on the electrical properties of the resulting semiconductor/polymer diodes.

Junctions were formed by preparing a layer of polyacetylene, $(CH)_x$, on Si (3.15 Ω cm (100) n-Si, or 2.45 Ω cm (111) p-Si, etched three times in 48% aqueous HF for 20 s, ref. 4) through the ring-opening metathesis polymerization of cyclooctatetraene[5]. Neat cyclooctatetraene was polymerized under nitrogen using the soluble tungsten carbene catalyst $W(CHC(CH_3)_3)(NR)(OR_F)_2$, where R is 2,6-$C_6H_3(CH(CH_3)_2)_2$ and OR_F is $OCCH_3(CF_3)_2$ (ref. 6). The catalyst (8 mg) was added to the liquid monomer (100 µl), and the desired substrate coated with the resulting solution. After 10 min, an ~300-µm-thick film of polymer formed on the Si surface. The excess monomer was removed under vacuum and the polyacetylene made conductive[7] either by exposure to iodine vapour followed by vacuum pumping, producing $(CHI_{0.17})_x$, or by exposure to a potassium benzophenone ketyl solution in 2-methyl tetrahydrofuran, producing $(CHK_{0.06})_x$. Conductivities of these doped films were determined by standard four-point-probe methods; for $(CHI_{0.17})_x$, $\sigma = 150\,\Omega^{-1}\,cm^{-1}$, and for $(CHK_{0.06})_x$, $\sigma = 50\,\Omega^{-1}\,cm^{-1}$. Electrical contact to the polyacetylene was made by immersing a gold wire in the cyclooctatetraene; the wire became encapsulated by the polymer during polymerization, and was held in place satisfactorily by the set polyacetylene film. Separate measurements determined that the doped polymers made low-resistance ($<50\,\Omega$) contacts to gold. The polyacetylene devices were always handled initially in a dry nitrogen atmosphere to avoid the decomposition reactions known to occur on exposure of doped and undoped polyacetylene to air[8]. When devices were to be handled in air, degradation was prevented by sealing the junctions with a coating of epoxy. Using these procedures, no degradation of electrical properties was noticed until at least two weeks after junction formation.

Previous work has shown that the rectification properties of n-Si and p-Si junctions with conventional metals are relatively insensitive to the contacting metal, even though such behaviour is not expected based on the ideal Schottky model of these junctions[1-3]. Figure 1 shows schematic energy diagrams for several types of Si junctions. In an ideal system, the position of the equilibrium Fermi level (E_F) in the Si is directly related to the work function of the contacting metal. The Si-band edge energies can be determined from the known values of the electron affinity of Si (4.05 V) and the Si band gap (1.12 eV)[2]. As can be seen in Fig. 1*b*, *c*, a change in the work function of the contacting phase should change the equilibrium position of the Si Fermi level. But for Si contacts to conventional metals, the Fermi level is found to be pinned to a small energy region. Metals ranging from copper to platinum (with work functions from 4.0 to 5.5 eV) all form low-barrier ohmic contacts to p-Si and rectifying junctions (with barrier heights of 0.7–0.9 eV) to n-Si, whatever the actual value of the metal work function (see Fig. 1*a*). High-barrier-height contacts to p-Si and low-barrier-height contacts to n-Si, as depicted in Fig. 1*c*, cannot be formed from direct Schottky contacts to these metals. Typical data for Si/Au junctions obtained in our laboratory are in agreement with these non-ideal properties (Fig. 2). The insensitivity of current density as a function of voltage (J–V properties) for a large variation in metal work function is termed Fermi-level pinning, and has generally been ascribed to the tendency of silicon to form metal silicides with most metals of interest[2,9-11]. The metal silicide layer then determines the J–V properties of the semiconductor/metal contacts, and precludes manipulation of the device properties by changing the metal.

Figure 2*a* displays the J–V properties of p-Si/$(CHI_{0.17})_x$ and p-Si/$(CHK_{0.06})_x$ contacts. A unique feature of a conducting polymer such as polyacetylene is that it can be doped with either oxidants or reductants, making it a material with a variable work function. The high-work-function[12] $(CHI_{0.17})_x$ overlayer yields a poorly rectifying contact to p-Si, as expected from the ideal band-equilibration model presented in Fig. 1*b*. Similarly, the low-work-function[13] $(CHK_{0.06})_x$ overlayer yields excellent rectifying properties on p-Si (Fig. 1*c*). A quantitative analysis can be made using the diode equation $J = J_0[\exp(qV/AkT) - 1]$, where k is the Boltzmann constant, T is the temperature, J_0 is the exchange (saturation) current density, A is the diode quality factor, and q is the electronic charge[2,3]. Better rectification corresponds to lower values of J_0 and ohmic contacts result from high J_0 values. The p-Si/$(CHK_{0.06})_x$ contact yielded a J_0 value of 6×10^{-7} A cm^{-2}, which is at least three orders of magnitude lower than the J_0 of conventional p-Si/metal contacts (typically 10^{-3} A cm^{-2}; see Fig. 2 and ref. 2). In contrast, the p-Si/$(CHI_{0.17})_x$ contact exhibited a J_0 value of 3×10^{-3} A cm^{-2}, providing a low-barrier contact.

The model in Fig. 1 also predicts that complementary behaviour should be observed with n-type Si as the semiconductor, where low-work-function contacts should yield ohmic contacts and high-work-function materials should provide rectifying diodes. Figure 2*b* displays the data for n-Si/$(CHI_{0.17})_x$ and n-Si/$(CHK_{0.06})_x$ contacts. The J_0 values for three separate n-Si/$(CHI_{0.17})_x$ samples were 2.9×10^{-8}, 2.7×10^{-8} and 2.4×10^{-8} A cm^{-2}, whereas the n-Si/$(CHK_{0.06})_x$ system had a poorly rectifying, ohmic J–V response, with a total resistance of 200 Ω. The range of J_0 values obtainable on conventional n-Si/metal interfaces is limited to about three orders of magnitude, whereas the J_0 values reported here span six orders of magnitude for the n-Si/$(CH)_x$ system (iodine and potassium dopants) and are in qualitative accord with the predictions of Fig. 1.

The behaviour of these conducting polymer/inorganic semiconductor interfaces is significant because it shows that restrictions on Schottky barrier devices can be overcome by using conducting organic polymer contact layers. Further, the ability to manipulate the properties of these interfaces by changing the polymer dopant allows switchable devices to be made. For instance, we have switched a device from rectifying to ohmic and back to rectifying behaviour by alternate iodine/alkali metal/iodine treatment of the $(CH)_x$ layer of an n-Si/$(CH)_x$ contact. A few previous studies of polyacetylene contacts to inorganic semiconductors have been reported using

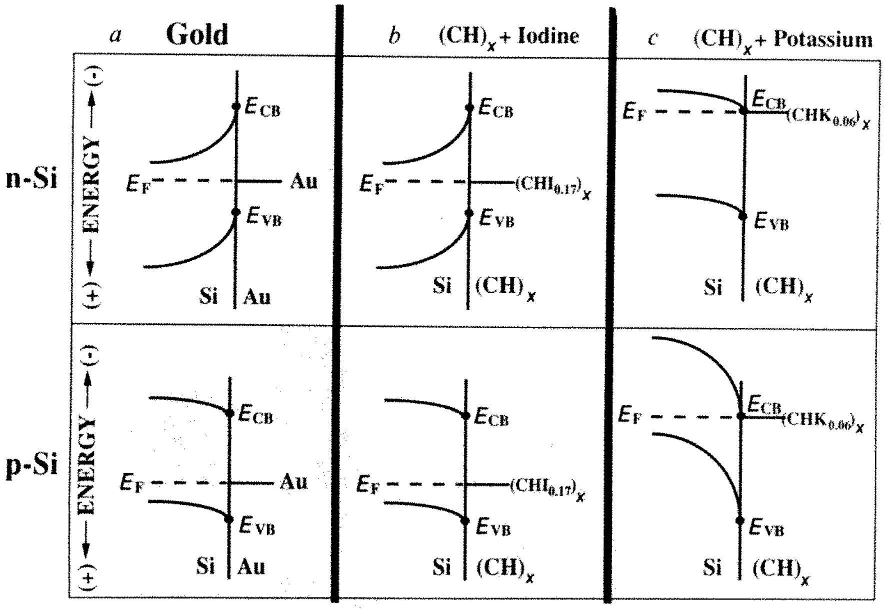

FIG. 1 Band-bending diagrams for n- and p-Si contacts to Au and $(CH)_x$ (I_2- and K-doped). The barrier height on n-Si is defined as the energy difference between the conduction-band edge, E_{CB}, of the Si and the equilibrium Fermi level, E_F of the contact. The barrier height for a p-type semiconductor is equal to $E_{VB} - E_F$. The energies of the conduction band (4.05 eV) and the valence band (5.17 eV) are obtained from experimental values of the electron affinity and band gap of Si. See text for discussion.

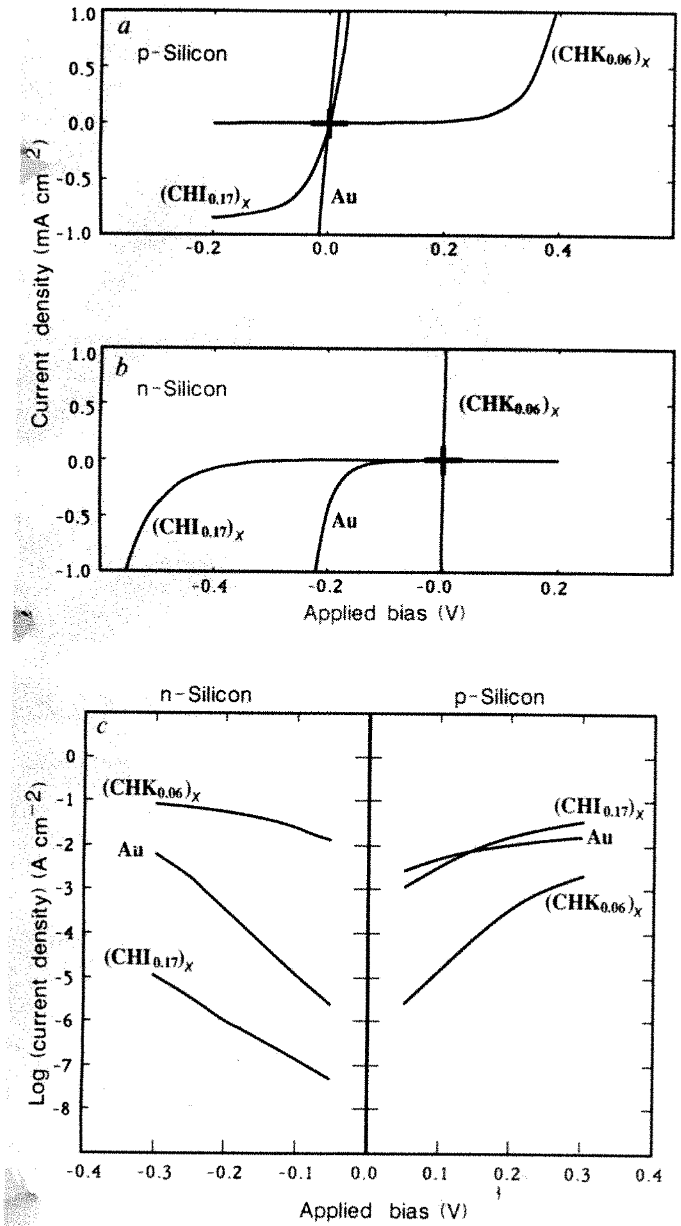

FIG. 2 Current density as a function of voltage for (*a*) p-type and (*b*) n-type Si/polyacetylene interfaces. The rectification characteristics change with the identity of the polymer dopant. The current-voltage curves for Si/Au diodes (typical of most Si/metal contacts) are added for comparison. The intercept (at zero applied bias) of the plot of log (current density) against forward bias (*c*) is a measure of the exchange (saturation) current density (J_0) for each interface. Curvature in the log plots at higher current densities arises from series-resistance effects in the diodes. The ratios of forward to reverse rectification (at ±1 V) are: p-Si/$(\mathrm{CHK}_{0.06})_x = 6\times10^3$, p-Si/$(\mathrm{CHI}_{0.17})_x = 2\times10^2$, n-Si/$(\mathrm{CHI}_{0.17})_x = 5\times10^4$. The diode quality factors are: n-Si/$(\mathrm{CHI}_{0.17})_x = 1.8$, p-Si/$(\mathrm{CHK}_{0.06})_x = 1.2$.

polyacetylene prepared by the Shirakawa route[14-16], although the ability to manipulate the device properties by varying the polymer dopant was not described. The Shirakawa polyacetylene has been used in photoelectrochemical cells to inhibit photocorrosion and to mediate charge transport between the semiconductor and a solution redox species[17], but the electrical properties of the isolated semiconductor/conducting polymer junction were not reported. New synthetic routes to polyacetylene have resulted in materials with superior processing and electronic characteristics. For instance, the recent fabrication by Burroughes *et al.*[18] of transistors and metal-insulator-semiconductor capacitors using polyacetylene as the active element relied on the Feast precursor method to generate homogeneous defect-free polyacetylene films[19,20]. Our work uses the recently developed liquid-phase ring-opening metathesis method of polymerizing cyclooctatetraene, which is more controllable than the gas-phase polymerization of acetylene[5]. This allows for uniform coverage of material over the semiconductor surface and for facile control of the morphology of the layer of doped organic polymer. The ability to prepare junctions with well defined electrical properties is a key step towards the development of polymer-based electronic devices. □

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Coherent structures at the ocean surface in convectively unstable conditions

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THE turbulent boundary layer at the ocean surface has some dynamical similarities to the atmospheric boundary layer[1-4]. The atmospheric turbulent boundary layer may exhibit not only random fluctuations but also spatially coherent, organized motion[5,6]. Thorpe[1] conjectured that such organized motion should also be found in the upper ocean boundary layer in convectively unstable conditions. Here I report on observations made in the tropical Atlantic Ocean which confirm this view. Horizontal temperature profiles obtained at a depth of 2 m at night revealed ramp-like structures. Vertical velocity profiles in the upper few metres of the ocean was determined using a free-rising profiler, and exhibited abrupt changes corresponding to sudden changes in temperature. These features are known[5,6] to be characteristic of spatially coherent, organized motions in turbulent boundary layers.

Laboratory experiments[7] in a coordinate system moving with the average convection velocity of coherent structures show that the typical organized motion in the unstratified turbulent boundary layer resembles an inclined three-dimensional horseshoe vortex. In stratified conditions the vorticity features are accompanied by density ones. Measurements[6] using a fixed probe in

TABLE 1 Observation of coherent structure in the upper ocean boundary layer

Run	Latitude	Date (1985)	Time (GMT)	U_0 (m s^{-1})	θ_0 (deg)	V_{10} (m s^{-1})	T_w (s)	H_w (m)	T_s (s)	H_s (m)	Q_R (W m^{-2})	Q_n (W m^{-2})	u_* (cm s^{-1})	T_* (°C)	L_* (m)	μ_3
							(*a*) Horizontal structure									
16 A, B	2° N	21 June	00:20–00:42	3.5		1*	ripples		5	1.0	0	−90	0.11	−0.049	−0.05	0.15, 0.43
16 C	2° N	21 June	00:42–00:52	3.5	0	1–3		<0.3	5	1.0	0					0.07
16 D, E, F	2° N	21 June	00:52–01:24	3.5	0	3.4	3	0.3	5	1.0	0	−140	0.38	−0.022	−1.3	−0.98, −1.00, −0.76
17 A, B, C	2–3° N	21 June	01:55–02:27	7.5	−55	3.0	3	0.3	5	1.0	0	−130	0.34	−0.023	−1.1	−0.41, −0.20, −0.[illegible]
13 A, B, C	2–1° N	20 June	14:18–14:50	7.5	37	3.0	3	0.3	5	1.0	840	−130	0.34	0.055	1.1	0.18, 0.18, 0.09
14 A, C, D	2–1 °N	20 June	15:11–15:22 15:32–15:54	7.5	40	5.0	3	0.5	5	1.5	880	−200	0.56	0.028	2.3	0.12, 0.14, 0.13
14 E, F, G	2–1° N	20 June	16:01–16:33	7.5	45	6.3	3	0.5	5	1.5	800	−190	0.71	0.020	5.3	0.15, 0.09, 0.21
							(*b*) Vertical profiles									
14–20	20° N	16 May	00:55–01:53	2.2		4.1	3	0.3	7	1.0	0	−180	0.46	−0.022	−1.9	
72–77	20° N	23 May	21:03–21:34	2.2		5.2	3	0.3	8	1.0	0	−200	0.59	−0.020	−3.6	

U_0 is the speed of probe relative to water, θ_0 the angle of ship's motion with respect to wind; V_{10} is the wind speed at a height of 10 m; T_w, T_s and H_w, H_s are the periods and the heights of wind waves and swell, respectively; Q_R is the solar insolation, Q_n the heat loss from the ocean surface owing to latent, sensible and long-wave radiative heat fluxes; $u_*=(\tau/\rho)^{1/2}$ is the friction velocity, where τ is the wind stress and ρ is the density of water; $T_*=Q_0/xu_*c_p\rho)$ is the friction temperature, where Q_0 is the heat flux in the upper layer of the ocean defined as $Q_0=Q_n+(1-A)(1-f(z_0))Q_R$, $A=0.05$ is the albedo of the ocean surface, f is a function describing volume absorption of solar radiation in the upper layer of the ocean, $z_0=2$ m, $x=0.4$ is von Karman's constant and c_p is the specific heat; $L_*=u_*^3/(x\gamma gQ_0/c_p\rho)$ is the Monin–Oboukhov length scale, where γ is the coefficient of thermal expansion of water and g is the acceleration due to gravity. Friction velocity was calculated as: $u_*=(\rho_a/\rho)^{1/2}C_{10}^{1/2}V_{10}$ where ρ_a is the density of air and C_{10} is the drag coefficient calculated in ref. 15, 1.04×10^{-3}. The latent and sensible heat fluxes were calculated from the air–ocean observations using standard bulk formulation[16]. Radiation fluxes were measured directly. Volume absorption of solar radiation was calculated as in ref. 17. Parameters of surface waves were obtained visually by a meteorologist.

* wind direction was unstable.

the convectively unstable atmospheric boundary layer show that the temperature has a characteristic ramp profile—a gradual rise followed by a relatively sharp decrease downstream—which leads to a skewed temperature derivative[1]. The sharp change of temperature occurs when a frontal interface of the horseshoe vortex passes the temperature probe. The temperature ramps in the atmospheric boundary layer were suggested[5] to be a signature of organized large-scale motion. In the surface ocean only the coherent structures of day-time stably stratified conditions have so far been observed[1,8].

An underwater probe[9] was mounted on the bow of the RV *Akademic Kurchatov* at a depth of 2 m (Fig. 1). The probe measured the temperature and conductivity fluctuations ahead of the moving vessel. The uncertainty on the temperature measurement was ~±0.001 °C; the response time was 0.1 s. An acceleration sensor registered vertical motions of the probe caused by the pitching of the vessel. The acceleration signal was doubly integrated and processed by a digital high-pass cosine filter with a 0.02-Hz cut-off frequency (to avoid low-frequency trends) and therefore gives information about the depth variation of the probe with respect to a mean hydrostatic level of the ocean.

At the probe mount the radius of curvative of the vessel's hull is ~10 cm, with a frontal angle of 16° (Fig. 1). In front of a moving sphere the streamlines are not appreciably disturbed at the distances greater than ~3 radii[10]. Owing to a 'knife-edge' hull, the *Akademic Kurchatov* produces practically no bow wave ahead of itself, as confirmed by photographs. No turbulent or wave perturbations from the vessel's body are therefore expected in the measurement zone.

A free-rising profiler[4] (Fig. 1) measured vertical profiles of conductivity and the vertical component of velocity fluctuations in the frequency range 2–250 Hz. The vertical velocity of the profiler was 2.2 m s^{-1}. Temperature profiles were calculated from the conductivity profiles neglecting salinity variation[4,11]. The uncertainties on the temperature and velocity fluctuations were ~0.001 °C and 1 mm s^{-1} respectively. The profiler tends to follow the wave-induced orbital motion in the long surface waves[4]. This reduces the influence of surface waves on turbulence measurements in the upper boundary layer of the ocean; the vertical profiles may be found relative to a moving coordinate system following long waves (details are given in ref. 4).

Measurements with the underwater probe were made at 37° W between 1° N and 20° N in June 1985. At several points along this section the measurements were also made into the wind direction. Here I ignore data obtained when it was raining or in regions of oceanic fronts.

At night the temperature fluctuations in the upper ocean appreciably exceeded the noise level of the probe only at wind speeds ≲5–7 m s^{-1}. In particular the measurements made in the

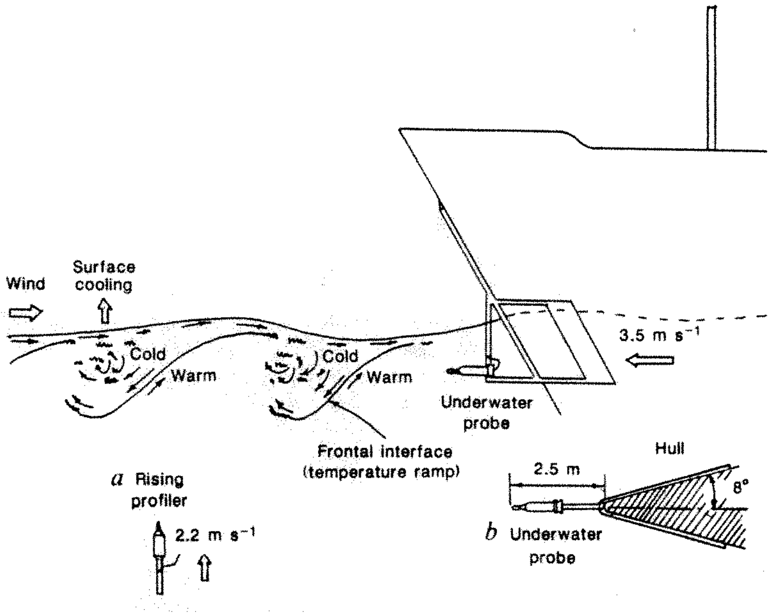

FIG. 1 Schematic representation showing spatially coherent organized motion in the upper ocean boundary layer and experimental techniques and devices used in measurements. *a*, Free-rising profiler, *b*, Top view at the probe mount (2 m below).

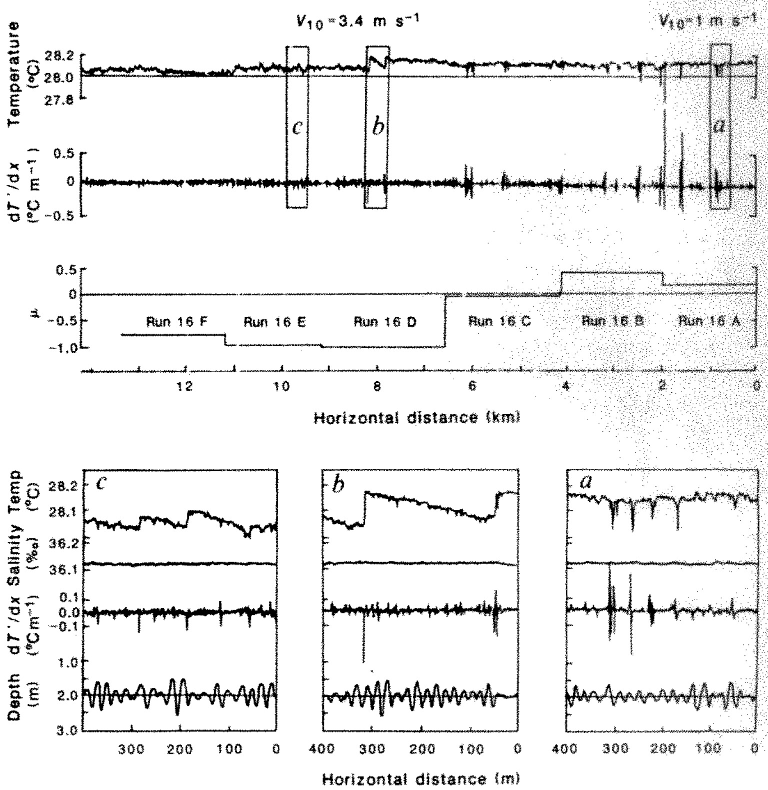

FIG. 2 Top, records of temperature (T), fluctuation-temperature derivative (dT'/dx) and skewness (μ_3) of the derivative obtained at 2 m depth in the early morning of 21 June (run 16 in Table 1). The graphs are plotted from right to left. Below, the marked regions (a, b and c) from above are shown in more detail along with the appropriate salinity records and depth variations of the probe relative to the mean hydrostatic level of the ocean, calculated from the accelerometer signal.

night of 20/21 June met this requirement (runs 16 and 17 in Table 1). Run 16 shown in Fig. 2 was made with the vessel heading into the wind at a speed of 3.5 m s^{-1}, after diurnal thermocline deepening. The real thickness of the seasonal mixed layer was ~50 m. The initial part of the record (run 16 A, B) obtained in almost calm weather conditions was typical of buoyancy-driven convection ($z_0/L_* \approx -40$, where z_0 is the mean depth of the probe, L_* is the Monin-Oboukhov scale), isolated narrow regions of cooler water of ~1-m scale (Fig. 2a) being ascribed to intersection of so called 'thermals' previously observed on the vertical temperature profiles in the convectively unstable upper boundary layer of the ocean[11,12] and of a lake[13]. A ramp-like structure with negative skewness μ_3 of the temperature derivative appeared in the temperature record (run 16, D-F in Fig. 2) after the wind speed increased. The value of $z_0/L_* \approx -1.3$ indicated that in this case the shear could have an important role.

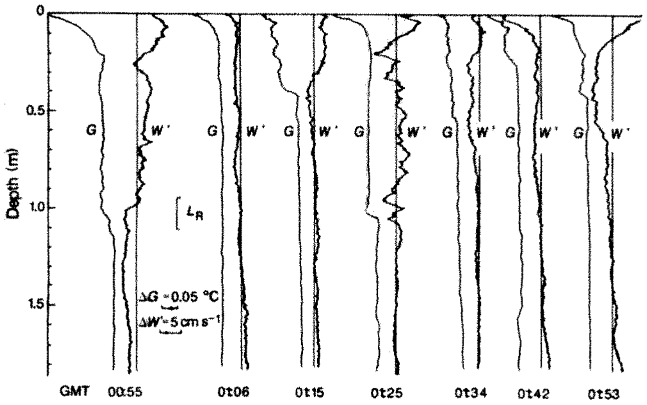

FIG. 3 Vertical profiles of temperature (G, calculated from conductivity profiles assuming constant salinity) and vertical component of velocity fluctuation (W') in the upper ocean in convectively unstable conditions. A positive $\Delta W'$ indicates a positive velocity change of the flow along the profiler. The vertical length scale (L_R) represents the relaxation time of velocity sensor, calculated using $L_R = W_0/(2\pi f_1) = 17.5$ cm, where $W_0 = 220$ cm s^{-1} is vertical velocity of profiler, $f_1 = 2$ Hz is lower frequency limit of the sensor.

Patterned on Fig. 4 of ref. 8, which shows processes of the vertical transport in a stably stratified shear boundary layer of the upper ocean, Fig. 1 reconstructs water circulation in unstably stratified conditions. As the ship moves upwind the ramps are observed as the probe passes from the 'warm water' to the 'cold water' side of the inclined sharp interfaces (Fig. 1). The ramps on the temperature record shown in Fig. 2 (run 16, D-F) are also consistent with this sketch.

The skewness of the temperature derivative for run 16 D-F falls in the range −0.7 to −1.0. In the convectively unstable atmospheric boundary layer the skewness in the presence of the coherent structure is near the latter value[14]. As expected, the mean value of μ_3 measured in the night-time convectively unstable conditions depended on the direction of the ship's motion relative to the wind (compare run 16, D-F with run 17, A-C in Table 1). During the day the μ_3 became positive (see runs 13 and 14), the values being near those obtained previously[1,8] in day-time stably stratified upper ocean in similar wind conditions and towing angles.

The vertical velocities measured by the free-rising profiler complement the ship-borne observations. This instrument was deployed 91 times in wind speeds of 2-7.5 m s^{-1} in the vicinity of 20° N, 37° W. Thirteen measurements (7 on 16 May and 6 on 23 May) were made under weather conditions very similar to those during the runs on 20-21 June (see Table 1). Figure 3 shows a series of seven successive measurements of temperature and vertical velocity fluctuations made by the free-rising profiler in the early morning of 16 May. The time interval between successive measurements was ~10 min and the vessel wind drift during this period was several tens of metres. The investigated coherent structure had time and spatial scales of the same order, and therefore no determinable connection between any of the series profiles is expected.

The abrupt change of temperature and vertical velocity component at the depth of 1.08 m observed on the profiles obtained at 01:25 GMT (Fig. 3) can be ascribed to vertical intersection of a frontal interface (see the sketch on Fig. 1 showing eddies and frontal interfaces in the upper ocean boundary layer). The temperature change at this interface was $\Delta\theta \approx -0.05$ °C, the appropriate change of the vertical velocity being $\Delta W \approx 2.5 \text{ cm s}^{-1}$. The sign of the vertical velocity change at 1.08 m depth indicated that the cold water was moving along the interface away from the ocean surface. This coincides with water circulation induced by the large-scale eddies in the upper ocean boundary layer (Fig. 1).

Similar profiles obtained at 00:55 GMT (Fig. 3), but having the opposite sign of the vertical velocity change at 0.95 m depth and the same sign of the temperature change as the profiles discussed above, may be interpreted as a result of intersection of the region where the cold water was moving along the frontal interface toward the ocean surface (see Fig. 1).

The profiles obtained at 01:06 GMT have no substantial changes either in temperature or in vertical component of velocity. These profiles can be interpreted as referring to a space between the large-scale eddies. The other four pairs of profiles shown in Fig. 3 apparently are concerned with cases when the profiler passes through less pronounced regions of the organized vortex structure. Vertical profiles obtained on 23 May had similar temperature and velocity features. □

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Consumption of atmospheric methane by tundra soils

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EMISSION of methane from tundra soil contributes about 10% of the global atmospheric methane budget[1]. Moreover, tundra soils contain 15% of global soil carbon[2], so the response of this large carbon reservoir to projected global warming[3,4] could be important. Coupled biological models[3–6] predict that a warmer climate will increase methane emission through increased rates of methanogenesis. Microbial oxidation of methane is, however, a possible control on emissions that has previously been overlooked. Here we report the results of field and laboratory experiments on methane consumption by tundra soils. For methane concentrations ranging from below to well above ambient, moist soils were found to consume methane rapidly; in non-waterlogged soils, equilibration with atmospheric methane was fast relative to microbial oxidation. We conclude that lowering of the water table in tundra as a result of a warmer, drier climate will decrease methane fluxes and could cause these areas to provide a negative feedback for atmospheric methane.

Methane is one of several radiatively active trace gases undergoing an atmospheric concentration increase of 1% yr^{-1} or more[7,8], and has the potential to cause climate change through atmospheric warming[9–11]. Global methane budgets balance net CH_4 sources to the atmosphere against the atmospheric photochemical sink, using ^{14}C and ^{13}C contents, and isotope fractionation factors as constraints[1,12] on the source terms. Other methane sinks (consumption by soils and marine sediments) are incorporated in the net source terms and are not explicitly identified in these budgets. Some climate models incorporating greenhouse gas increases predict higher temperatures and drier summer conditions for high northern latitudes[13–15]. Coupled biological models predict increased CH_4 emission as a result of increased rates of methanogenesis[3–6], but CH_4 oxidation was neglected in these models. Net consumption of atmospheric CH_4 has been reported in some soils of temperature forests[16–18] and swamps[19], tropical forests[18,20–22], savannah[23] and tundra[24,25]. The areal extent of the soil CH_4 sink is poorly understood, but it is estimated to account for <1–58 Tg yr^{-1}, or up to 11% of the global photochemical sink[16,17,21].

Our observations were made in a moist tundra meadow on Unalaska Island, Aleutian Islands, adjacent to Skan Bay (53° N, 167° W). Plant cover was a continuous mat of mosses invaded by lichens, cottongrasses (*Eriophorum* sp.), low-lying ferns and dwarf shrubs (*Vaccinium* sp). The air temperature ranged between 5 and 8 °C; the soil temperature was 7 °C and uniform in the upper 15 cm.

We collected syringe samples of gas from three static chambers at 0.25-h intervals and analysed them by gas chromatography[26] on board the RV *Alpha Helix*. The chambers were fitted with a capillary bleed to equalize pressure following sampling. Chamber measurements yield net fluxes—in the measurements reported here, the methane concentration decreased with time, indicating that consumption exceeded production in the moist

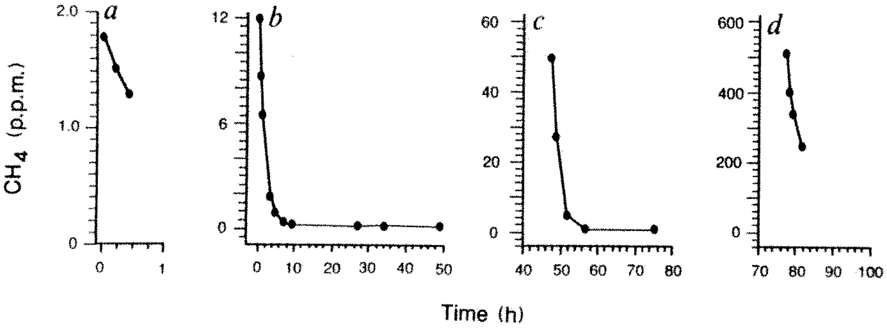

FIG. 1 Consumption of headspace methane in a field experiment using a static chamber. *a*, consumption of atmospheric CH_4 to sub-ambient levels on 8 October 1987. *b–d*, Results of subsequent experiments (9–12 October 1987) in which the headspace of the same chamber was adjusted to initial CH_4 concentrations of 10, 50 and 550 p.p.m., respectively. Chamber temperatures differed from ambient temperatures by <2 °C during the experiment.

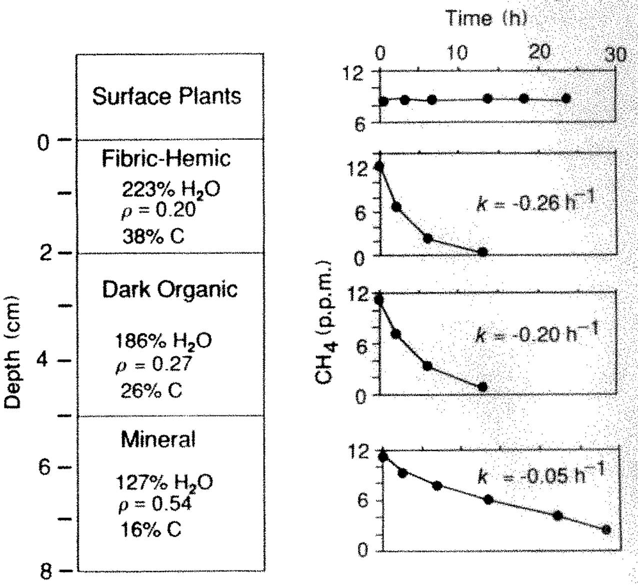

FIG. 2 Soil characteristics and depth distribution of CH_4 consumption in a soil core. Maximum consumption rates occur in the top 5 cm of the core. No CH_4 was consumed by the vegetation. Water content ([wet weight/dry weight] − 1), organic content ([loss on ignition]/[dry weight]) and bulk density (ρ represents [oven-dried mass]/[field volume] in g cm^{-3}) are given for each of the soil units.

soils studied. In all cases, the concentrations eventually level off to a small but non-zero value. Figure 1 shows the results of an experiment on one of two chambers where atmospheric methane (~1.7 p.p.m.) was consumed, and subsequent experiments where the chamber headspace was adjusted to initial CH_4 concentrations of 10, 50 and 500 p.p.m. by injection of millilitre quantities of a 95% Ar:5% CH_4 mixture. Headspace concentrations in chambers with 10 p.p.m. CH_4 became sub-ambient in 4-7 h and decreased to 0.14 p.p.m. in as little as 8 h.

Soil cores from the same site were collected in plastic tubes (6.7-cm inner diameter, 2-mm wall) for laboratory studies of CH_4 oxidation and the depth distribution of CH_4 oxidizing potential. We avoided sample disturbance by cutting around the tube perimeters with a serrated knife as the tubes were inserted. The cores collected were 2-12 cm long, and showed a 2-cm fibric-hemic layer below the surface vegetation followed by a 3-cm dark organic-rich layer that graded into a weathered mineral horizon extending below the sample (Fig. 2). These cores were capped at the base and were placed in ~1-l Mason jars, the lids of which were equipped with valves and a septum. The jar headspaces were maintained at constant pressure by introducing ambient air following sample removal; this headspace dilution was negligible. Because only the soil surface was in contact with the jar atmosphere, the cores in jars were physically equivalent to the field chambers. The ratios of headspace volume to soil surface area for the chambers and jars agreed to within 5%, so CH_4 uptake kinetics for both are comparable. The response of whole cores to CH_4 additions at ambient air temperatures was repeatable and identical to the field chambers—the first-order rate constants for methane consumption in both were ~−0.25 h^{-1} and headspace methane concentrations as low as 0.7 p.p.m. were observed in 6-13 h.

Methane consumption by core segments representing the various soil horizons was measured in jar experiments using initial headspace CH_4 concentrations of ~10 p.p.m. Microlitre quantities of the same Ar:CH_4 mixture that was used in the chamber experiments were added to the jars. The results are shown in Fig. 2. The surface vegetation consumed no CH_4. Methane was consumed by all soil layers, with the highest rates in the 0-2 cm and 2-5 cm layers. A portion of each core segment was autoclaved; these samples consumed no CH_4 in 24 h, indicating that CH_4 consumption is biologically mediated.

We performed a tracer experiment to determine the products of methane oxidation and their distribution in soils. Two cores with nearly identical methane uptake kinetics (cores C and E) were used in this experiment, one to follow CH_4 decreases and the other to follow changes in headspace $^{14}CH_4$ and $^{14}CO_2$. The experiment was conducted in the dark to prevent photosynthetic uptake of $^{14}CO_2$. The headspaces of the jars containing both cores were adjusted to an initial CH_4 concentration of ~10 p.p.m., but stable CH_4 was added to core C whereas core E received CH_4 containing $^{14}CH_4$ (2.8×10^8 d.p.m. cm^{-3}; d.p.m., disintegrations per minute). Core E was sampled at time intervals based on decreases in the stable CH_4 concentration of core C. Headspace samples (10 cm^3) were injected into a metal and glass stripping/oxidation line[27] and were carried in a helium flow through bubbler traps and a quartz combustion tube filled with CuO heated to 800 °C. The $^{14}CO_2$ was trapped in 1N NaOH before combustion and the $^{14}CH_4$ was trapped in Woeller's solution[28] as $^{14}CO_2$ following combustion. The jar headspaces were maintained at constant pressure by introducing ambient air following sample removal. The results are corrected for dilution (1.6% per sample). The experiment was terminated after 11 h when the CH_4 concentration reached 0.4 p.p.m. and 95% of the added $^{14}CH_4$ had been consumed. The remaining headspace $^{14}CH_4$ and $^{14}CO_2$ was recovered with a series of helium flushes; the jar and core were frozen and the ^{14}C incorporated in the core was assayed by dry combustion of freeze-dried homogenized samples of core segments.

Figure 3 shows the results of the tracer experiment. Core C showed a decrease in headspace CH_4 that parallels the $^{14}CH_4$ decrease in core E. The headspace $^{14}CO_2$ activity indicated that part of the consumed $^{14}CH_4$ was retained by the core. We accounted for 96% of the $^{14}CH_4$ added to core E; 46% of this was respired as $^{14}CO_2$ and 54% was recovered by dry combustion. The fraction recovered by dry combustion represents $^{14}CH_4$ assimilated into microbial biomass, organic matter and inorganic matter. We made no attempt to independently assay the inorganic fraction; these soils are carbonate-free and soil pH values of 5.7 suggest that this fraction is small.

Interpretation of experiments involving amended atmosphere requires an understanding of the timescale of equilibration between the soil and headspace for added gases. The equilibration time was evaluated for core E with a relaxation technique[29] that involved inhibiting methane oxidation and following equilibration of added CH_4. Acetylene, a well-known inhibitor of CH_4 oxidation[30], was added to the headspace (30 p.p.m. final concentration) before sectioning core E for freeze-drying and dry combustion. Methane was then added and headspace concentrations were measured at 1-min intervals until the changes could not be resolved (6 min). This approach shows that equilibration is a two-step process: a fast ($k = -1.54$ min^{-1})

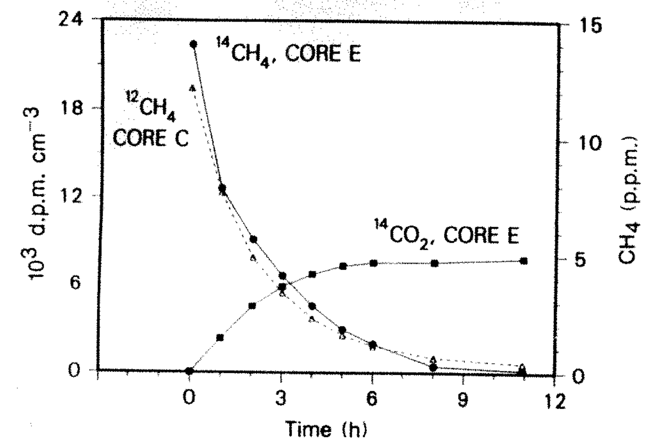

FIG. 3 $^{14}CH_4$ oxidation as a function of time. This jar experiment was conducted on 12 October 1987, using two cores with almost identical CH_4 oxidizing characteristics. The headspace of core C was amended with CH_4 and was used to guide sampling core E, which was amended with $^{14}CH_4$.

process, presumably involving the porous surface material or large voids, followed by a slower ($k = -0.75\ \text{min}^{-1}$) process involving more compact material or adjacent smaller voids. These first-order equilibration or relaxation constants show that equilibration is much faster than biological consumption ($k = -0.004\ \text{min}^{-1}$). Thus, CH_4 consumption is not controlled by transport in tundra soils. The initial samples in the chamber, jar and tracer experiments (taken 15 min after adjusting initial concentrations in the chamber and jar experiments; 5 min after tracer addition) contain no bias owing to incomplete equilibration.

The ability of these soils to respond to methane increases without a lag and to consume a wide (thousand-fold) range of concentrations with no indication of saturation (all follow first-order consumption kinetics) indicates that they could be an important sink. These methane consumption measurements were made at the low end (7 °C) of the reported temperature range (2–30 °C). Methane consumption rates of 2.7 mg m^{-2} d^{-1} for our unamended chambers lie in the middle of reported ranges (0.2–4.2 mg m^{-2} d^{-1})[16-25], indicating that methane oxidation can be important in cold soils. Final methane concentrations as low as those observed in our chamber and jar experiments (0.1–0.4 p.p.m.) have not been reported in previous chamber experiments, but similar concentrations have been observed in soils[16]. Static chambers are rarely allowed to 'run down', so the high final concentrations in previous studies can be expected when static chambers are used for only short periods to avoid disturbing sub-surface gradients[31]. Our results demonstrate that soil oxidation of atmospheric CH_4 is microbially mediated and point to a population capable of oxidizing CH_4 at concentrations ten times lower than ambient atmospheric concentrations. Kinetic properties of cultured methanotrophs are not consistent with growth using atmospheric CH_4 as the sole carbon source, implying a threshold for growth well above atmospheric levels[32]. Mixotrophic growth, co-oxidation by non-methanotrophs such as ammonium-oxidizing bacteria, or induction of high-methane-affinity enzyme systems are processes that may be important in maintaining these low CH_4 concentrations[30,32,33].

These measurements have implications important to tundra systems under the projected warmer drier conditions. The subsurface CH_4 oxidizing activity is important in controlling upward CH_4 fluxes at sites where vascular plants are absent. Vascular plants transport CH_4 and effectively bypass the CH_4 oxidizing zone. Tussock and low-shrub tundra (35–50% vascular plant cover) are expected to show larger consumption effects than wet meadow tundra (80–90% vascular plant cover)[26]. Our previous observations at relatively wet permanent sites have not shown net oxidation[26]. Methane fluxes from tundra[34] and swamp[19] sites, however, are positively correlated with the level of the water table, and the 20% of the stations occupied during a high-latitude transect study[24], all with a lowered water table, had zero or negative CH_4 fluxes.

Consumption of atmospheric CH_4 by soils depends on transport to zones of consuming activity. Transport in waterlogged tundra soils is by aqueous molecular diffusion. Waterlogged tundra sites have a methane consumption maximum centred near the water table (the oxic/anoxic boundary), sustained by upward diffusion of dissolved CH_4. By contrast, transport in dry tundra soils is by gas-phase diffusion (10^5 times faster than aqueous), so porous low-resistance tundra soils do not limit the supply of methane. Expected consequences of atmospheric warming (such as a lowered water table, increased seasonal thaw depth, or permafrost melting) will increase the vertical extent of oxidized tundra soil and enhance oxidation of CH_4. Our measurements demonstrate that the oxidation of atmospheric CH_4 occurs in oxic soils and is not limited by diffusion in non-waterlogged soils, so a negative feedback on atmospheric CH_4 concentration is possible. Furthermore, the CO_2 produced by oxidizing CH_4 is 20 times less effective as a greenhouse gas[8]. □

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Uranium and ^{10}Be enrichments by fluids in Andean arc magmas

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VOLCANIC rocks from subduction settings frequently contain ^{10}Be enrichments that have been attributed to sediment subduction[1-6], and are often characterized by (^{238}U/^{230}Th) ratios greater than unity, in contrast to mid-ocean-ridge and ocean-island basalts[7,8]. Here we report such an excess of ^{238}U over ^{230}Th in historic lavas from the Southern Volcanic Zone of Chile (33–42° S). The greatest uranium excess is found south of 39° S, where ^{10}Be/^{9}Be ratios are highest and the trench is filled with sediment. These features suggest that both U and ^{10}Be enrichments result from preferential partitioning of U and Be into a fluid phase during dehydration of the recently subducted sediments. The presence of strong ^{238}U–^{230}Th disequilibria in basaltic magmas of the Southern Volcanic Zone suggests that the timescale for dehydration, melting and eruption in this arc is probably less than ~20,000 yr.

Andean Southern Volcanic Zone (SVZ), where subduction-related volcanism has occurred continuously since the Jurassic, is a single arc segment underlain by continental crust (Fig. 1). North of 37° S, the crust ranges in age from late Palaeozoic to Triassic and its thickness increases sharply from 35 km to 55–60 km between 34 and 36° S (ref. 9). South of 37° S, the crust is of Mesozoic to Cenozoic age and has a relatively uniform thickness of 30-35 km until 40° S where it thickens slightly to ~40 km (ref. 10). Throughout the SVZ the geometry and rate of subduction and the distance of the volcanic front from the trench remain nearly constant, although in the northern part between 33-37° S the age of the subducting crust is ~40 Myr and the trench is sediment-poor, whereas in the southern part between 37 and 42° S the age of the subducting crust is ~18 Myr and the trench is filled with sediment[11]. The absence of a significant accretionary prism inboard of the trench indicates that sediments are subducted beneath the arc[12].

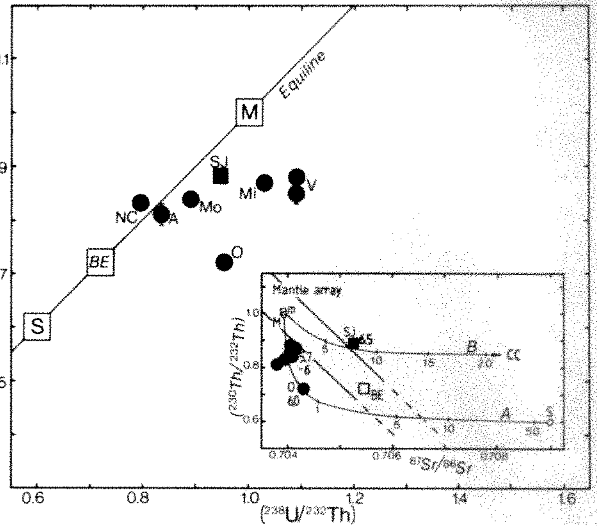

FIG. 2 Data for the SVZ volcanoes in the (^{230}Th/^{232}Th)–(^{238}U/^{232}Th) isochron diagram. Samples in U–Th radioactive equilibrium would plot on the equiline. NC, Nevados de Chillan; A, Antuco; Mo, Mocho; SJ, San Jose; O, Osorno; Mi, Mirador; V, Villarrica. BE is the 'Bulk Earth' value[18]; M, mantle of ocean-island-basalt (OIB) type; S, sedimentary component. The SVZ mantle source on the equiline can have been modified by addition of ancient sediments to an OIB-like mantle. Inset, Th–Sr isotope diagram of the same samples. The numbers adjacent to data points indicate the δ^{18}O values of the samples. Curve A: Hypothetical model explaining the modification of the mantle sources under the SVZ by mixing between an OIB-like mantle source (M), with (^{230}Th/^{232}Th)=1.0, ^{87}Sr/^{86}Sr=0.7039, Th=0.04 p.p.m., Sr=20 p.p.m., and a sedimentary component (S) with (^{230}Th/^{232}Th)=0.6 (inferred from Th/U ratio), ^{87}Sr/^{86}Sr=0.709, Th=15 p.p.m., and Sr=300 p.p.m. (from ref. 19). Curve B: Crustal contamination model for the San Jose magma: A mantle melt (m), having (^{230}Th/^{232}Th)=1.0, ^{87}Sr/^{86}Sr=0.7039, Th=0.5 p.p.m., Sr=400 p.p.m., assimilates Palaeozoic–Triassic crustal rocks (CC) with (^{230}Th/^{232}Th)=0.8, ^{87}Sr/^{86}Sr=0.770, Th=10 p.p.m., and Sr=100 p.p.m. (from ref. 9). The numbers on curves A and B refer to the percentage of contamination by sediments and continental crust, respectively.

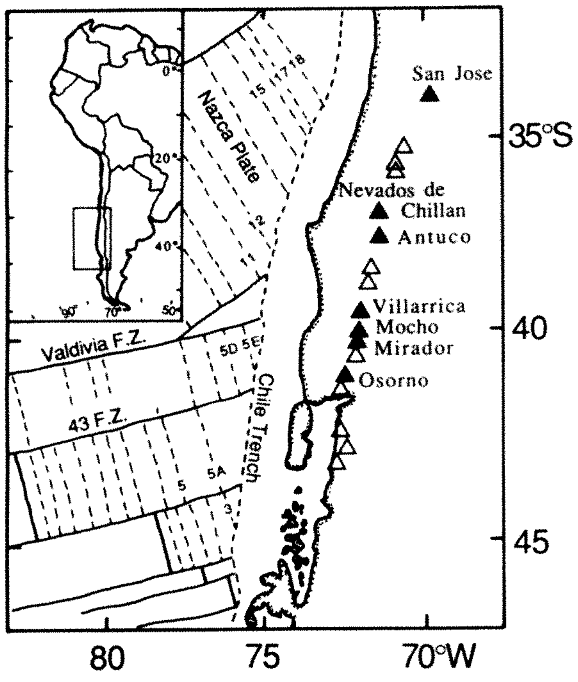

FIG. 1 Map of a part of the Southern Volcanic Zone of the Andes showing the volcanoes discussed. Nazca plate features are taken from ref. 11.

Our samples are lavas from eight historic eruptions at seven volcanic centres between 33 and 42° S (Fig. 1). The samples are calc-alkaline, high Al basalts and basaltic andesites, with two andesites and a dacite included in the suite. Only one volcano, San Jose is situated to the north of 37° S and, therefore, has been constructed on a thicker and older crust than the other volcanoes. For the six volcanic centres south of 37° S, medium-K lavas consist of anhydrous mineral assemblages composed predominantly of olivine and plagioclase, with occasional clinopyroxene in mafic rocks, and plagioclase and two pyroxenes in intermediate lavas, whereas the lava from San Jose is a high-K hornblende andesite. The analytical results of ^{238}U–^{230}Th disequilibria and Sr and O isotopes together with previous ^{10}Be measurements[2] are reported in Table 1.

When plotted on the isochron diagram (Fig. 2) the SVZ data form a nearly horizontal array extending right from the equiline. The (^{238}U/^{230}Th) ratios range from 0.96 to 1.33, whereas (^{230}Th/^{232}Th) ratios vary only between 0.81 to 0.88, with the exception of the Osorno basalt, which has the lowest measured (^{230}Th/^{232}Th) ratio of 0.72. Intermediate and evolved lavas are similar to mafic ones in terms of (^{230}Th/^{232}Th) ratios. Despite

TABLE 1 Isotope data

Latitude	Volcano	Date of eruption	Rock type	SiO_2 (wt%)	U (p.p.m.)	Th (p.p.m.)	Th/U	$\left(\frac{^{238}U}{^{232}Th}\right)$	$\left(\frac{^{230}Th}{^{232}Th}\right)$	$\left(\frac{^{238}U}{^{230}Th}\right)$	^{10}Be ($\times10^6$ atom g^{-1})	$^{10}Be/^9Be$ ($\times10^{-11}$)	$^{87}Sr/^{86}Sr$	$\delta^{18}O$ (‰SMOW)
33°45′ S	San Jose, 281284-4	1800s	And	62.5	3.24	10.4	3.21	0.945	0.88 ± 0.01	1.07	1.4	1.0	0.70527 ± 2	6.5
36°45′ S	N Chillan, 060376-1	1930s	Dac	65.6	2.29	8.73	3.82	0.795	0.83 ± 0.01	0.96	1.8	1.4	0.70395 ± 2	5.9
37°22′ S	Antuco, 051177-7	1853	Bas	50.2	0.402	1.46	3.64	0.834	0.81 ± 0.02	1.03	1.0	2.3	0.70379 ± 2	5.9
39°25′ S	Villarrica, 101275-1	1971	Bas	52.5	0.447	1.24	2.78	1.09	0.85 ± 0.02	1.28	2.1	5.3	0.70404 ± 2	5.8
39°25′ S	Villarrica, 191284-1	1984	Bas	52.3	0.429	1.19	2.77	1.09	0.88 ± 0.01	1.24			0.70403 ± 2	5.7
40°50′ S	Mocho, 220281-1	1864	And	58.8	0.873	2.98	3.41	0.889	0.84 ± 0.01	1.06	1.3	2.0	0.70410 ± 2	6.0
40°30′ S	Mirador, 220479-3	1979	Bas	53.5	0.236	0.697	2.95	1.03	0.87 ± 0.01	1.19			0.70413 ± 2	5.9
41°10′ S	Osorno, 090185-1H	1834	Bas	53.0	0.303	0.963	3.18	0.954	0.72 ± 0.01	1.33	1.4	3.7	0.70429 ± 2	6.0

U and Th contents are determined by isotope-dilution mass spectrometry. Ratios in parenthesis are activity ratios. The relative 1σ errors on ($^{238}U/^{232}Th$) and ($^{238}U/^{230}Th$) ratios are estimated at 0.59 and 2% respectively. The errors on ($^{230}Th/^{232}Th$) are 1σ statistical α-counting errors. Reproducibilities (1σ) for measurements are better than ±20% for $^{10}Be/^9Be$ ratio. The Sr isotope ratios have bee normalized to a value of $^{86}Sr/^{88}Sr = 0.1194$ and $^{87}Sr/^{86}Sr = 0.71022$ for the NBS-987 standard; the errors are 2σ in-run errors. Reproducibilities for $\delta^{18}O$ are better than 0.2‰ and the NBS-28 gav $\delta^{18}O = 9.6‰$ ($\delta^{18}O = [(^{18}O/^{16}O)_{sample}/(^{18}O/^{16}O)_{SMOW}] - 1$, SMOW is standard mean ocean water). wt%, weight per cent.

the near constancy of Th isotope ratios, the Th/U ratios vary considerably (2.77-3.82) but with no clear geographical trend along the strike of the arc.

The ($^{230}Th/^{232}Th$) ratios of SVZ lavas are too low to be inherited from a mantle source such as those giving rise to mid-ocean-ridge basalt (MORB) or ocean island basalt (OIB), yet the similarity of the ($^{230}Th/^{232}Th$) ratios of most SVZ lavas favours an origin from a rather homogeneous reservoir. Crustal contamination would hardly result in the constant ($^{230}Th/^{232}Th$) ratios in different lava types from distant volcanoes. The low Th isotope ratios also preclude any major involvement of altered basalts from the subducted oceanic crust. Such basalts would be enriched in uranium and therefore would have high ($^{230}Th/^{232}Th$) ratios after radioactive equilibrium had been reached. The low ($^{230}Th/^{232}Th$) may be a characteristic of a subduction-modified mantle wedge underlying most part of the studied area. As sediments have been subducted since the Jurassic, the mantle sources have probably been affected by mixing with this component. Pelagic and terrigenous sediments often have high Th/U, and hence low ($^{230}Th/^{232}Th$) ratios (see Fig. 2). Thus sediment injection may have lowered the ($^{230}Th/^{232}Th$) of the sub-arc mantle, and this can explain the location of most data below the 'mantle array' in the Th-Sr isotope diagram (Fig. 2, inset). Mixing of a sedimentary component in the mantle source will also lead to higher $^{87}Sr/^{86}Sr$ ratios as schematically illustrated by the mixing curve. Most samples from 36 to 42° S have $^{87}Sr/^{86}Sr$ ratios around 0.7040, except for the basalt from Osorno, which has a slightly higher $^{87}Sr/^{86}Sr$ ratio and lower ($^{230}Th/^{232}Th$) ratio. Only a small contribution (<1%) of the sedimentary component is necessary to explain the thorium and strontium isotope data. Thus the $\delta^{18}O$ values will not be significantly affected in this process: they range from 5.7 to 6.0‰ and do not differ from normal mantle values. Only the high-K andesite from San Jose, north of 37° S, has higher $\delta^{18}O$ (6.5‰) and $^{87}Sr/^{86}Sr$ (0.7053): the magma in this volcano has probably been contaminated by old and ^{18}O-rich continental crust (Fig. 2, insert).

Previous results indicate that the relatively constant Pb isotope ratios in the SVZ lavas[9,10] can be explained neither by simple mixing of mantle material and presently subducted sediments, nor by a contamination from the continental crust[9]. But the introduction into the mantle of old and radiogenic sediments during the 200 Myr of convergence in this zone can produce the Pb isotope signature of the SVZ lavas. Radiogenic sediments could have been formed by erosion of the South America Precambrian craton and deposited by the rivers in the Pacific Ocean before the Andean orogeny. Another possible mechanism is the erosion of the Palaeozoic/Precambrian continental edge[12]. Thus the Pb isotope data does not contradict the model of a mantle source progressively modified by subducted sediments.

The limited variation of the different isotope ratios in the SVZ lavas suggest that this contribution is relatively constant between 37 and 41° S and that the mantle wedge is well mixed and isotopically homogeneous. Further south, however, Osorno has a distinctive Th and Sr isotope composition, indicating a greater contamination of its mantle source by ancient sediments, perhaps related to a thinner mantle wedge in this area.

These time-integrated modifications of the mantle wedge do not explain the origin of the ^{238}U enrichment over ^{230}Th, which obviously requires a recent (<300,000 yr) event in the mantle source. This uranium enrichment is not restricted to oceanic island arcs as previously suggested[8,13]. The fact that many subduction zone volcanoes lie on the right of the equiline in the isochron diagram is usually interpreted as reflecting the preferential uranium enrichment of the mantle wedge by slab-derived fluids before or during melting[14,15]. In this context it is particularly appropriate to compare ^{238}U-^{230}Th disequilibria and ^{10}Be results.

The presence of cosmogenic ^{10}Be in many lavas from young volcanic arcs and its total absence in MORB or OIB lavas

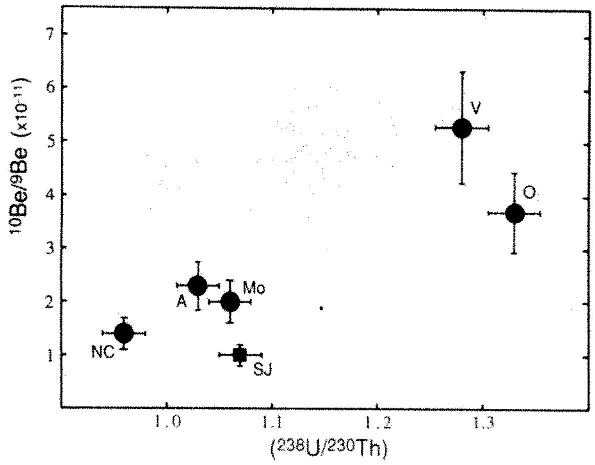

FIG. 3 Variation of $^{10}Be/^9Be$ with ($^{238}U/^{230}Th$) in the SVZ lavas. Abbreviations as in Fig. 2. Assuming a ^{10}Be-free mantle source, the extrapolation of this correlation to $^{10}Be/^9Be = 0$ can give the ($^{238}U/^{230}Th$) ratio of the primary melt before fluid addition. If this value is constant for all the SVZ primary magmas, then the maximum proportion of U added by fluids to the primary melt can be calculated for each volcano; it amounts to ~35% for Villarrica.

supports the proposition that subducted pelagic sediments, younger than 10 Myr, contribute to the formation of arc magmas[1-5]. Concentrations of ^{10}Be for the historic SVZ lavas analysed are given in Table 1 and these values (1.0-2.1×10^6 atom g^{-1}) are significantly higher than mantle or lower crustal values ($\ll 1 \times 10^6$ atom g^{-1}). They have been interpreted to reflect the incorporation of a subducted component during or before magma generation[2,5,6]. The ^{10}Be and (^{238}U/^{230}Th) ratios in SVZ lavas are compared in Fig. 3. Despite rather large analytical uncertainties in the ^{10}Be measurements, a fair correlation is apparent; the lavas having the highest uranium enrichment (south of 39° S) also exhibit the highest ^{10}Be/^{9}Be ratios. These results are in contrast to those from the New Britain arc[16,17], where highest ^{10}Be/^{9}Be are associated with highest (^{230}Th/^{232}Th) ratios, but are not well correlated with uranium excesses. The correlation between ^{10}Be/^{9}Be and (^{238}U/^{230}Th) in the SVZ samples is even better if we exclude the contaminated San Jose sample. The high ^{10}Be/^{9}Be ratios of the Villarrica and Osorno lavas can be attributed to the injection in the mantle of a greater amount of recent sediments. Indeed the extension towards the continent of the Valdivia Fracture Zone and 43° Fracture Zone (Fig. 1) which may act as sediment traps, pass directly beneath these volcanic centres. The absence of any correlation between Pb isotopes[9,10] and ^{10}Be/^{9}Be ratios confirms that, in contrast with ^{10}Be/^{9}Be ratios, the arc lead budget is the result of long-term modification of the mantle source by sediment subduction.

The correlation between ^{10}Be/^{9}Be and (^{238}U/^{230}Th) strongly suggests that a common process is responsible for the U and ^{10}Be enrichment. Boron probably accompanies ^{10}Be and U because ^{10}Be/^{9}Be and B/^{9}Be ratios are well correlated for SVZ lavas[6]. This process is most probably the recent dehydration of subducted sediments. The correlation also suggests that the U and ^{10}Be enrichments are related to the abundance of fluids and, therefore, to the amount of recently subducted sediments. The sediment subduction does not inhibit large earthquakes as evidenced by the great 1960 earthquake, which had its epicentre in this region of the Andes.

If the U and ^{10}Be enrichment is indeed the result of fluid transfer to the zone of magma generation in an otherwise homogeneous mantle source, then the nearly horizontal array in the isochron diagram (Fig. 2) has an age significance: it shows that the time interval between the fluid induced U-Th fractionation in the mantle and eruption of the magmas is ≤20,000 yr. Further discussion on the relative contributions of old and recent subducted sediments, mantle wedge and continental crust requires further data on other SVZ volcanoes, but our study demonstrates that U and ^{10}Be enrichments in the SVZ magmas are produced by the introduction in the mantle of fluids derived from sediments presently subducted under the Andes. ☐

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Observations of seismic reflectors in the lower lithosphere beneath the Skagerrak

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THE lithosphere—the rigid outer shell of the Earth—includes both the crust and the upper mantle. In the past decade, the complex continental crust and underlying shallower mantle lithosphere have been extensively studied using reflection seismology, but the lower lithosphere has not been successfully imaged. Here we report the observation of continuous and virtually horizontal reflectors in the lower continental lithosphere down to depths of 100–110 km below the Skagerrak (Scandinavia). These are apparently not structurally associated with the crust, whereas previously reported mantle reflectors, such as those observed by the BIRPS group around the British Isles[1,2], are often located near large faults in the overlying crust. Like the crust, the mantle lithosphere shows a seismically transparent upper and a reflective lower part. Thus, the processes that form seismic reflectors seem to have an affinity for ductile rheological environments in the mantle as well as in the crust. Our observations are consistent with a model consisting of a mechanically strong upper lithosphere, underlain by a thermal boundary layer which separates it from the convecting asthenosphere.

The roughly horizontal mantle reflectors were recorded on a 100-km-long experimental ultra-deep seismic reflection profile (OG-13) that was part of a comprehensive deep seismic survey (1,730 km; 16-s record length) conducted by the research vessel *Mobil Search* in the Skagerrak in 1987 (Fig. 1)[3]. A 40-s record length was used with a 4.5-km-long streamer having 180 hydrophone groups. The energy source was a 9,000-in^3 airgun array. As reflected energy is low for the large depths involved, much effort was put into processing refinements. After extensive experimentation, we chose a BIRPS processing scheme[4] except that we used a weighted trace mix in the common offset domain before stacking. The latter process gave a significant improvement of the data quality in the lower parts of the section as compared to more conventional extended-array simulations.

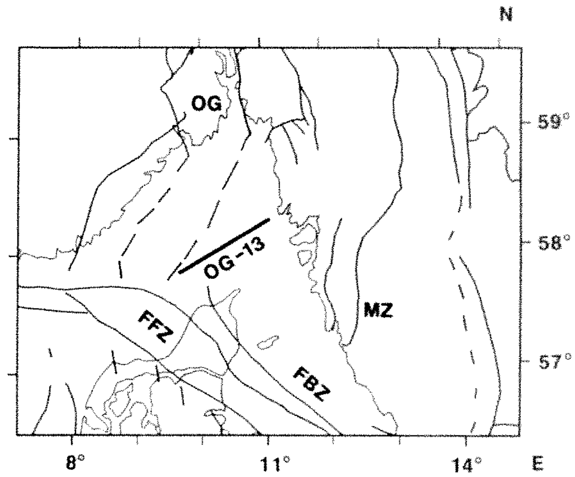


FIG. 1 Map showing the location of the deep seismic profiling line OG-13 in the Skagerrak. The principal geological features are outlined; OG: Oslo Graben; FBZ: Fennoscandian Border Zone; FFZ: Fjerritslev Fault Zone; MZ: Mylonite Zone.

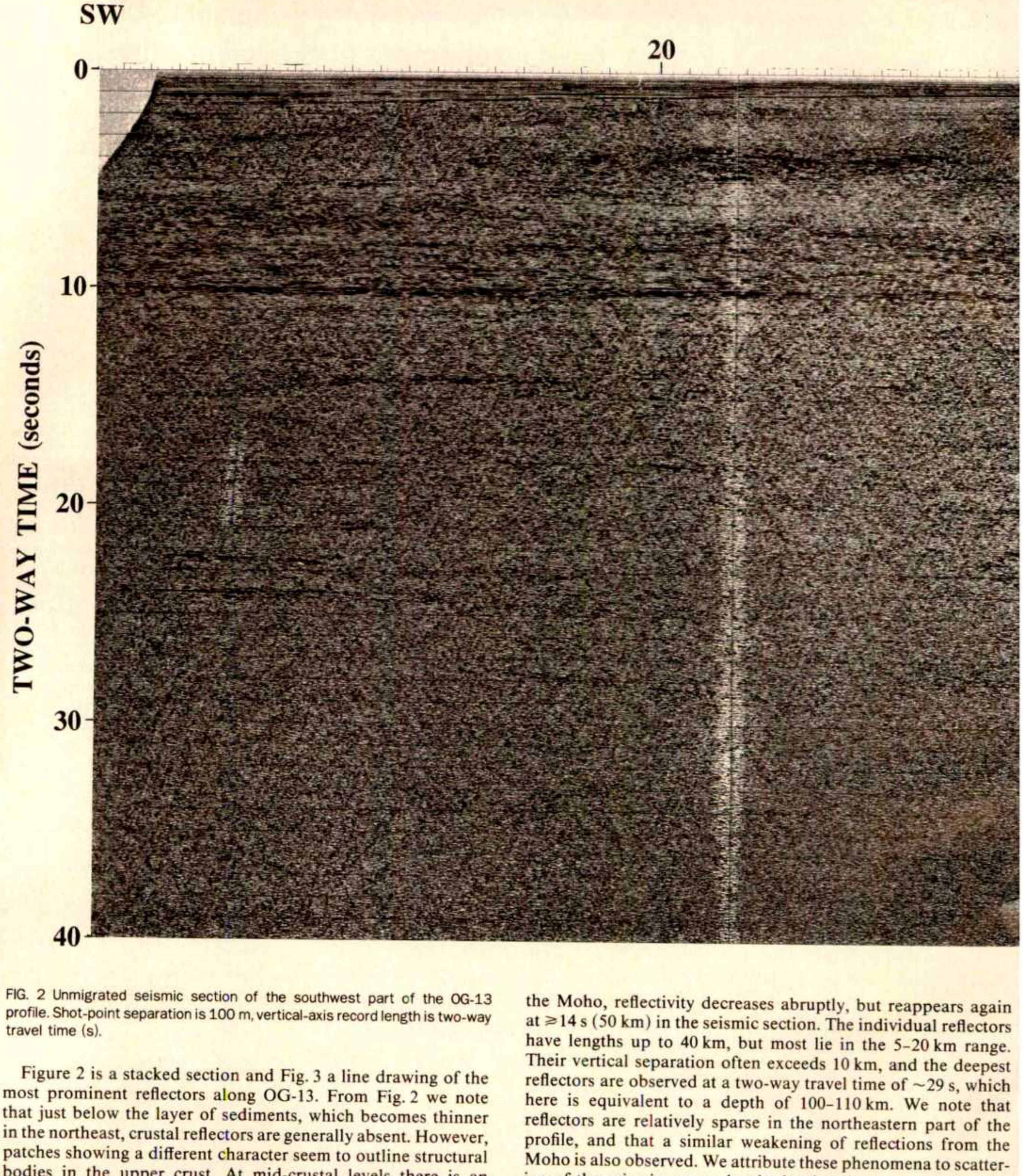
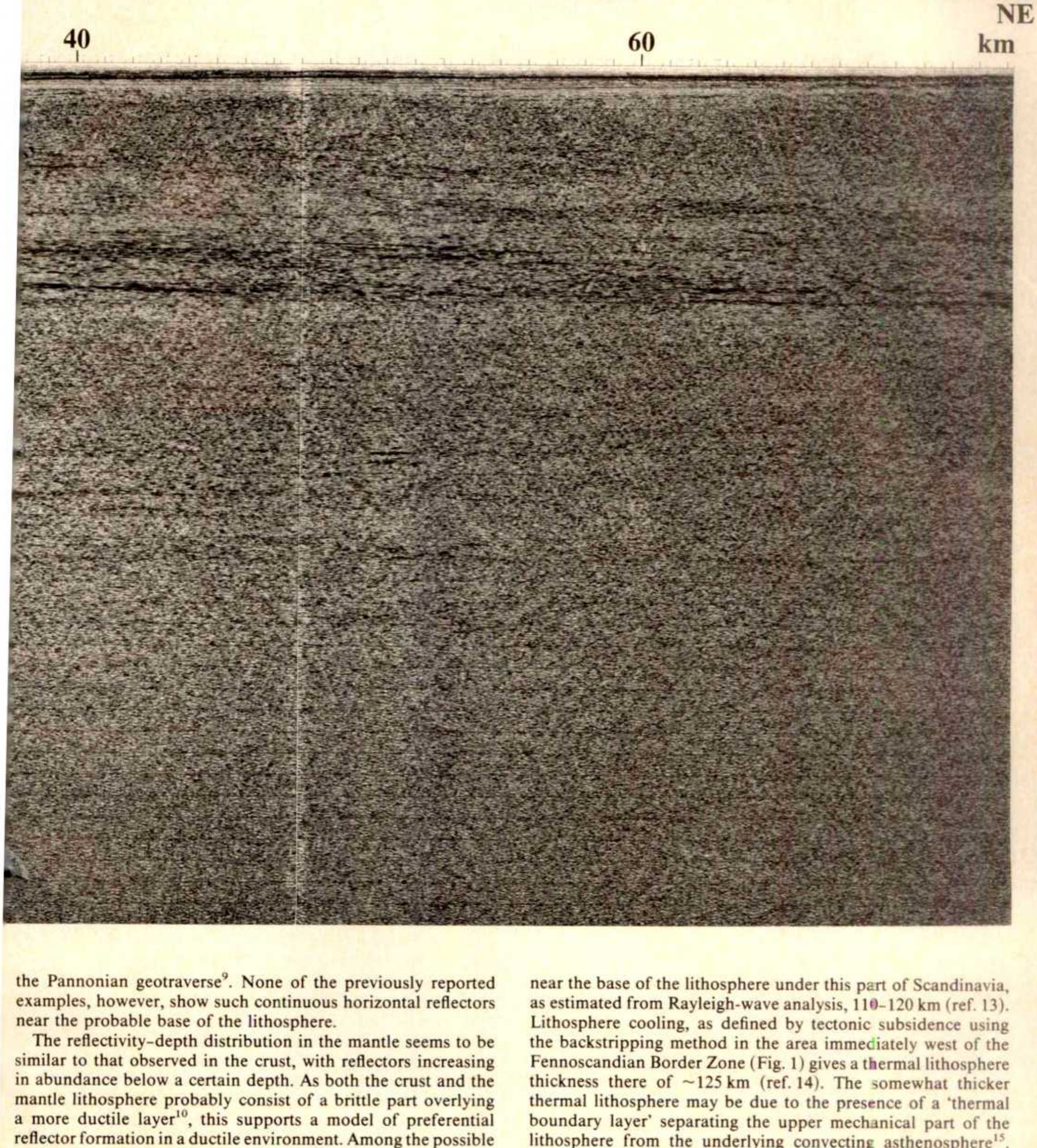


FIG. 2 Unmigrated seismic section of the southwest part of the OG-13 profile. Shot-point separation is 100 m, vertical-axis record length is two-way travel time (s).

Figure 2 is a stacked section and Fig. 3 a line drawing of the most prominent reflectors along OG-13. From Fig. 2 we note that just below the layer of sediments, which becomes thinner in the northeast, crustal reflectors are generally absent. However, patches showing a different character seem to outline structural bodies in the upper crust. At mid-crustal levels there is an increase in reflectivity, which may mark the transition from an brittle upper to a ductile lower crust[5,6]. Lower crustal reflectors with individual lengths of a few kilometres often form lineaments 20-30 km long. The crustal reflectors seem to exhibit a distinct pattern that dips and thins towards the northeast where the crust thickens towards the centre of the Fennoscandian Shield. The reflective lower crust is bounded below by the 'reflection' Moho, which is well defined as an almost continuous reflective band ~200-400 ms (0.7-1.5 km) wide. Closer inspection reveals that the Moho at 10-s two-way travel time (~30 km) is laminated with 3-6 parallel segments, each typically 1-4 km long. Below the Moho, reflectivity decreases abruptly, but reappears again at ≥14 s (50 km) in the seismic section. The individual reflectors have lengths up to 40 km, but most lie in the 5-20 km range. Their vertical separation often exceeds 10 km, and the deepest reflectors are observed at a two-way travel time of ~29 s, which here is equivalent to a depth of 100-110 km. We note that reflectors are relatively sparse in the northeastern part of the profile, and that a similar weakening of reflections from the Moho is also observed. We attribute these phenomena to scattering of the seismic energy by the hard and uneven sea floor in this part of the section, where a sediment cover is lacking. In the western half of the section a possible candidate for a converted S-wave reflection from the Moho is observed and has been modelled to have an arrival time of 14 s. We note, however, that a similar observation of a potential S-wave converted reflection from the Moho, the W-reflector on the DRUM profile off northwest Scotland[1], was later proved to be a P-wave reflection and not an S-wave artefact by the SLAVE two-ship experiment[7].

The existence of reflectors 5-20 km long in the lower mantle lithosphere have rarely been reported. Notable exceptions are the MOIST and DRUM profiles off northeast Scotland[1,8] and



the Pannonian geotraverse[9]. None of the previously reported examples, however, show such continuous horizontal reflectors near the probable base of the lithosphere.

The reflectivity-depth distribution in the mantle seems to be similar to that observed in the crust, with reflectors increasing in abundance below a certain depth. As both the crust and the mantle lithosphere probably consist of a brittle part overlying a more ductile layer[10], this supports a model of preferential reflector formation in a ductile environment. Among the possible explanations that have been put forward for the large impedance contrasts needed to form mantle reflectors are the presence of shear zones containing layers of hydrated minerals, caused by the passage of water, and layers of mafic igneous material due to partial melting[1]. Alternatively the presence of shear strain may favour layers with oriented olivine or large amounts of garnet[11,12]. Such hypotheses have not yet been substantiated by quantitative modelling, however, and no objective criteria exist to discriminate amongst them using seismic data. As the nature of mantle reflections remains an enigma, we refrain from further speculations on their character in the Skagerrak area.

The deepest reflections identified, at ~100–110 km depth, are near the base of the lithosphere under this part of Scandinavia, as estimated from Rayleigh-wave analysis, 110–120 km (ref. 13). Lithosphere cooling, as defined by tectonic subsidence using the backstripping method in the area immediately west of the Fennoscandian Border Zone (Fig. 1) gives a thermal lithosphere thickness there of ~125 km (ref. 14). The somewhat thicker thermal lithosphere may be due to the presence of a 'thermal boundary layer' separating the upper mechanical part of the lithosphere from the underlying convecting asthenosphere[15]. Whereas the mechanical part is capable of preserving structural and compositional features on a geological timescale, these are smeared out in the thermal boundary layer owing to episodic convection. Consequently, we do not expect to observe any reflectors there. It is also possible that the lack of observed reflectors is related to the limited dynamic range of the recording system and limited strength of the airgun signal, but it seems unlikely that the 'penetration depth' so defined should agree so well with lithosphere thickness as estimated by other and independent methods.

Our results indicate that the base of the lithosphere can be mapped using the seismic-reflection technique. Such results,

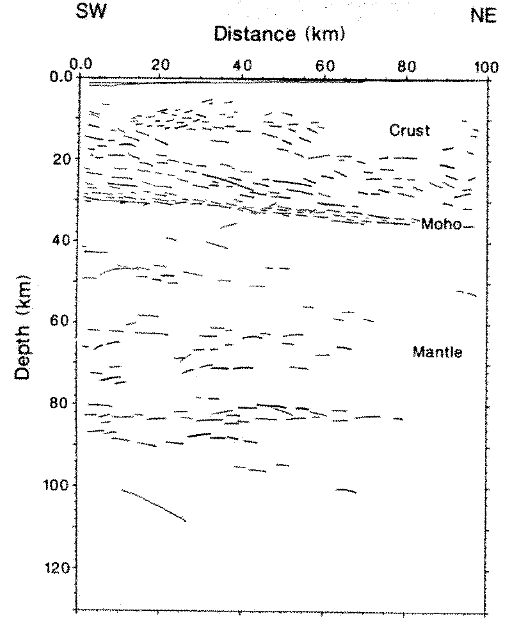


FIG. 3 Line drawing derived from the time-to-depth conversion of the unmigrated stack section using a simple velocity model.

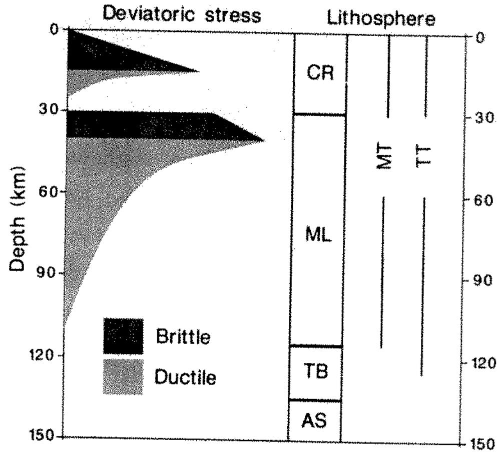


FIG. 4 Sketch of the lithosphere in the OG-profiling area. CR, crust; ML, lower lithosphere; TB, thermal boundary layer; AS, asthenosphere; MT, mechanical lithospheric thickness; TT, thermal lithospheric thickness. Deviatoric stress (relative scaling) calculated for a lithosphere rheology with quartz in the crust and olivine in the mantle lithosphere. The brittle and ductile regions are indicated. The thermally defined lithosphere is thicker than the one estimated from surface-wave analysis owing to the presence of the thermal boundary layer.

combined with data from other geophysical methods, may ultimately help us to improve our understanding of the nature of the lithosphere, its transition to the asthenosphere and the part it plays in plate tectonics. ☐

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Liquid immiscibility in a nephelinite–carbonate system at 25 kbar and implications for carbonatite origin

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MANTLE-DERIVED carbonate-rich melts may have an important role in mantle metasomatism[1,2], and may serve as parent liquids for crustal carbonatite magmas[3,4]. Experiments have shown that carbonatitic melts can be produced by partial melting of peridotite + CO_2 + H_2O above 22 kbar (ref. 3), and that silicate and carbonate liquids are immiscible between 2 and 15 kbar for a wide range of Ca/Na ratios[5–7]. We have determined the extent of silicate-carbonate liquid immiscibility at 25 kbar and 1,050–1,300 °C using mixtures of magnesian nephelinite, dolomite and sodium carbonate with and without water. In contrast to the low-pressure data, the two-liquid field at 25 kbar is restricted to more sodium-rich compositions, far removed from natural mantle melts. Our experimental results suggest that neither partial melting of carbonated peridotite, nor extensive fractional crystallization of silicate magmas at depths corresponding to 25 kbar, are likely to generate carbonatitic magmas by liquid immiscibility.

High-pressure melting studies in the system $CaO-MgO-SiO_2-CO_2$ have shown that initial liquids will be dominated by calcic dolomite (Ca/Mg > 1) and will have ~5–10 wt% silicate components[8,9]. In natural peridotites sodium will be strongly partitioned into this carbonatitic liquid[3,10]. None of these high-pressure melting studies, however, has demonstrated silicate-carbonate liquid immiscibility. Mixtures of kimberlite and calcite with and without H_2O did not show liquid immiscibility over a temperature and pressure range of 1,250–1,500 °C and 20–30 kbar (ref. 11). In vapour-saturated peridotite melting experiments above 31 kbar, Wallace and Green[3] reported possible silicate-carbonate liquid immiscibility at ~1,000 °C. Our study was undertaken to define the extent of silicate-carbonate liquid immiscibility over a range of temperatures and compositions at 25 kbar. Starting mixtures contained 30–50 wt% of a de-watered primitive nephelinite (Neph) from the Honolulu Volcanic field, Hawaii (68KEE-1)[12]. The remaining 50–70 wt% consisted of carbonate with ratios of dolomite (Dol) and Na_2CO_3 between 0 and 3. Mixtures with the same major-element compositions and containing 4.5 wt% H_2O were made up from combinations of oxides, carbonates and synthetic and natural minerals. Water was added in the form of $Mg(OH)_2$ and $Al(OH)_3$; 4.5 wt% was selected as a reasonable upper limit for mantle melts. A mixture consisting of 44 wt% nephelinite, 6 wt% Durango apatite and 50 wt% Na_2CO_3 was used to investigate the effects of P_2O_5 on immiscibility. All starting mixtures were dried for at least 1 h in a vacuum drying oven at 110 °C before being loaded into graphite crucibles, which were sealed inside platinum capsules. Although not rigorously buffered, experimental calibration sug-

TABLE 1 Coexisting liquids from selected 25-kbar experiments

Run	116		110		157	
T(°C)	1,250		1,150		1,050	
t(h)	10.2		9.2		6.0	
	Carb(10)*	Sil(9)	Carb(9)	Sil(11)	Carb(9)	Sil(9)
SiO_2	9.5 (1)†	33.7 (1)	6.64 (5)	34.2 (1)	19.5 (2)	30.9 (1)
TiO_2	1.02 (3)	2.42 (3)	0.68 (5)	2.68 (3)	1.49 (4)	2.07 (2)
Al_2O_3	2.1 (1)	9.39 (5)	2.4 (1)	10.4 (2)	6.1 (1)	10.33 (4)
FeO	6.15 (8)	10.36 (6)	5.04 (7)	10.3 (1)	7.4 (2)	8.12 (8)
MgO	6.60 (7)	7.5 (1)	3.2 (1)	5.21 (8)	7.3 (2)	6.88 (7)
CaO	11.98 (9)	6.00 (6)	17.78 (8)	8.21 (9)	9.4 (1)	5.91 (4)
Na_2O	60.6 (3)	29.8 (2)	55.8 (3)	26.7 (2)	47.5 (4)	34.6 (2)
K_2O	1.03 (2)	0.43 (2)	0.82 (3)	0.35 (2)	0.62 (2)	0.74 (2)
P_2O_5	1.09 (3)	0.30 (3)	7.32 (6)	1.92 (4)	0.65 (3)	0.41 (3)

* Number of analyses.

† Values in parentheses are standard deviations of the mean, that is, 9.5 (1) means 9.5 ± 0.1.

All liquids reported on a CO_2-free basis, normalized to 100%. Bulk compositions: 116 = 0.5Neph + $0.5Na_2CO_3$; 110 = 0.44Neph + 0.06Ap + $0.5Na_2CO_3$; 157 = 0.5Neph + $0.5Na_2CO_3$ + 4.5% H_2O. Abbreviations: Carb, carbonate liquid; Sil, silicate liquid; Neph, nephelinite; Ap, apatite.

gests that the oxygen fugacity in the graphite capsules was in the wüstite field[13].

Experiments were performed in a 1.27-cm piston-cylinder apparatus using calcium fluoride as a pressure medium. For the anhydrous starting compositions a series of timed experiments showed that a minimum of 6–8 h was required to approach two-liquid equilibrium. The hydrous experiments were run for 1–6 h. Products were polished without using water, and were analysed with a Camscan scanning electron microscope (SEM) fitted with an energy dispersive system. Quenched liquids and crystals were analysed using a beam current of 0.1 nA on brass. All of the liquids quenched to a fine intergrowth of carbonate and silicate material. The quenched liquids were analysed 9–11 times in raster mode; each analysis covered several hundred square micrometres. Liquid compositions from selected experiments are reported in Table 1.

Figure 1*a* shows the results of the 25-kbar nominally anhydrous experiments on the nephelinite–dolomite–sodium carbonate join. Liquids and starting compositions have been projected from CO_2 onto the plane ($SiO_2 + Al_2O_3 + TiO_2$)–($CaO + MgO + FeO^*$)–($Na_2O + K_2O$). This simple oxide wt% projection has been used extensively to display silicate and carbonate liquids at low to moderate pressures[6]. Also shown in Fig. 1*a* are the coexisting silicate and carbonate liquids in the systems sanidine–K_2CO_3 (ref. 14) and albite–Na_2CO_3 (D. Ellis and P.J.W., unpublished data), and the 5-kbar immiscibility field[6]. At 1,300 and 1,250 °C the two-liquid field is located in the sodium-rich portion of composition space. For both temperatures, the immiscibility field is broadest for mixture 1 (Dol/Na_2CO_3 = 0) and the field closes with increasing dolomite content. The field also widens with decreasing temperature.

At 1,250 °C both the mixture 1 (Dol/Na_2CO_3 = 0) and mixture 2 (Dol/Na_2CO_3 = 0.33) form two liquids. In both charges the silicate fraction forms a large spheroid (<800 μm in longest dimension) and is surrounded by carbonate melt. At 1,250 °C the silicate fraction in the mixture 1 experiment contains olivine

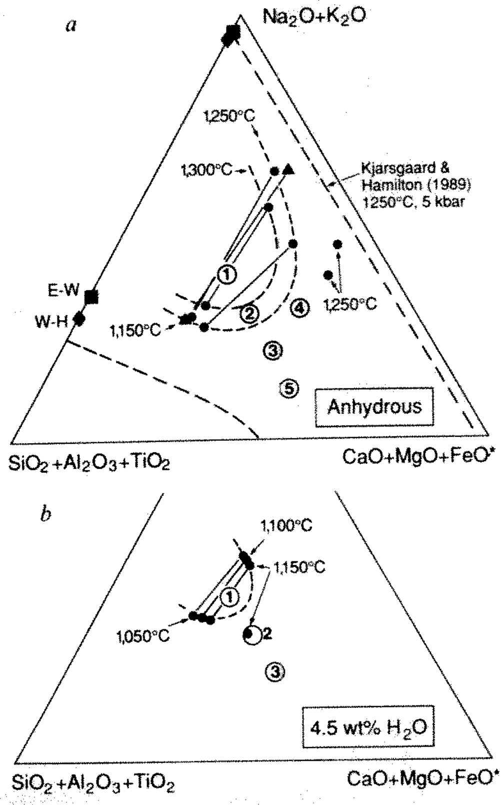

FIG. 1 Run products and starting compositions projected from $CO_2 \pm H_2O$ onto the plane ($SiO_2 + Al_2O_3 + TiO_2$)–($CaO + MgO + FeO^*$)–($Na_2O + K_2O$). FeO* is total Fe as FeO. Open circles denote starting mixtures: 1, 0.5Neph + $0.5Na_2CO_3$; 2, 0.5Neph + $0.375Na_2CO_3$ + 0.125Dol; 3, 0.5Neph + $0.25Na_2CO_3$ + 0.25Dol; 4, 0.3Neph + $0.35Na_2CO_3$ + 0.35Dol; 5, 0.5Neph + $0.125Na_2CO_3$ + 0.375Dol; abbreviations as in Table 1 and Dol, dolomite. *a*, Anhydrous experiments; closed symbols denote liquid compositions, triangles denote liquids from mixture 1 + 6% apatite experiment at 1,150 °C. Long dashes denote 5-kbar two-liquid field from Kjarsgaard and Hamilton[6]; W-H denotes experimental liquid from Wendlandt and Harrison[14]. E-W denotes experimental liquid from D. Ellis and P.J.W. (unpublished data). *b*, Results from experiments with 4.5 wt% H_2O; open circle denotes same starting mixtures as in *a* but containing 4.5 wt% H_2O; other symbols are the same as in *a*. Because of the closely spaced liquid compositions only one two-liquid field is shown.

the silicate fraction in the mixture 1 experiment contains olivine (86% fosterite, Fo_{86}) and aluminous spinel, and has ~34 wt% SiO_2 and ~30 wt% Na_2O (CO_2-free basis). SiO_2 and Na_2O values in the coexisting carbonate liquid are ~10 and 61 wt%, respectively. Olivine (Fo_{86}) and spinel are more abundant in the experiment with mixture 2 at 1,250 °C. Runs using mixtures 3 and 4 (both with $Dol/Na_2CO_3 = 1$) produced olivine, clinopyroxene, spinel and carbonated silicate liquids at 1,250 °C. Clinopyroxene dominates the crystalline assemblage in both runs and the resulting liquids are driven toward the (Na_2O+K_2O+K_2O)-(CaO+MgO+FeO*) join. Mixture 5 with $Dol/Na_2CO_3 = 3$ was largely crystalline at 1,250 °C, and the small pockets of liquid could not be analysed using SEM area scans.

The 1,150 °C tie line defines the extent of immiscibility in the mixture 1+6% apatite ($Dol/Na_2CO_3 = 0$). The silicate fraction contains olivine (Fo_{83}) and spinel. Although the two-liquid field has expanded slightly relative to the 1,250 °C boundary, this may simply reflect the 100 °C drop in temperature. Apatite does not seem to have a large effect on the size of the two-liquid field. For a variety of compositions, the silicate-carbonate immiscibility field extends from the (SiO_2+Al_2O_3+TiO_2)-(Na_2O+K_2O) join to close to the CaO+MgO+FeO* corner at crustal pressures (Fig. 1*a*). The decrease in the size of the anhydrous two-liquid field at 25 kbar compared to 5 kbar must reflect the increasing solubility of dolomite in silicate liquids with increasing pressure.

Figure 1*b* shows the results of the hydrous experiments. Runs using mixture 1+4.5% H_2O ($Dol/Na_2CO_3 = 0$) display immiscibility over the range of investigated temperatures (1,200-1,050 °C). At the lowest temperature the two liquids coexist with olivine. Experiments using mixture 2+4.5% H_2O ($Dol/Na_2CO_3 = 0.33$) contain a single liquid and minor olivine at 1,150 °C and a single liquid, olivine (Fo_{86}) and amphibole at 1,100 °C. Although we have not run experiments down to 950 °C, the solidus of fertile peridotite+vapour[3], we do not expect the two-liquid field to expand significantly between 1,050 and 950 °C. Further, below, 1,100 °C increasing crystallization of olivine+amphibole±clinopyroxene will drive residual liquids toward the (Na_2O+K_2O)-(CaO+MgO+FeO*) join and out of the immiscibility field.

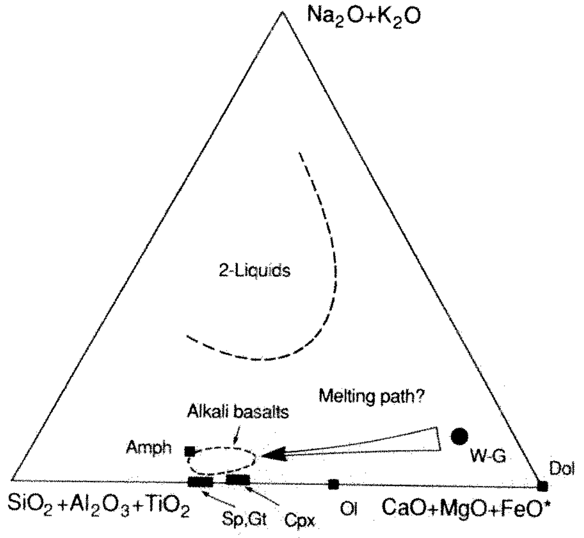

FIG. 2 Anhydrous 25-kbar two-liquid field and an inferred melting path for amphibole+dolomite peridotite projected onto the (SiO_2+Al_2O_3+TiO_2)-(CaO+MgO+FeO*)-(Na_2O+K_2O) plane. W-G denotes the carbonate liquid composition (Table 1, column 3)[3]; alkali basalt field[12,15]; squares and rectangles denote minerals that could crystallize from carbonate-rich liquids: olivine (Ol) and spinel (Sp) from this study, amphibole (Amph) from Wallace and Green[3], garnet (Gt) and clinopyroxene (Cpx) from M.B.B. and P.J.W. (unpublished data).

Silica, titania, alumina and iron are enriched in the silicate relative to the carbonate liquid (Table 1). The behaviour of titanium and aluminium indicates that phases such as ilmenite, spinel and garnet will have relatively low solubilities in carbonate melts. Thus partial melting of dolomite-bearing peridotite may be an effective mechanism for fractionating high-field-strength elements from other incompatible elements and light from heavy rare-earth elements. Calcium, sodium and phosphorus are enriched in the carbonate relative to the silicate liquids. In the anhydrous experiments K_2O is partitioned into the carbonate liquid, whereas in the runs with water K_2O is enriched in the silicate liquid.

Figure 2 shows the anhydrous 1,250 °C two-liquid field projected onto the (SiO_2+Al_2O_3+TiO_2)-(CaO+MgO+FeO*)-(Na_2O+K_2O) plane. The point labelled W-G is the carbonatitic melt produced by Wallace and Green[3] at 1,000 °C and 22 kbar and in equilibrium with olivine, orthopyroxene, clinopyroxene, amphibole, spinel and ilmenite. The region labelled alkali basalts includes a variety of nepheline normative liquids with Mg/(Mg+Fe*) ratios > 0.66 (refs 12, 15). These basaltic compositions are generally assumed to represent partial melts from a garnet peridotite source[16,17]. Figure 2 shows that a projected straight-line melting path from W-G to the alkali basaltic field does not intersect the two-liquid field. Note that the path is curved in CO_2 space because volatile contents decrease with increasing melt fraction; W-G contains ~45 wt% volatiles whereas most alkali basalts probably have <5 wt% H_2O and CO_2. There is no reason, however, to expect projected melting paths to curve toward the Na_2O+K_2O corner. Thus it seems unlikely that immiscible silicate and carbonate liquids can be directly produced by vapour-undersaturated partial melting of hydrated dolomite peridotite at pressures of ~25 kbar. Also plotted on Fig. 2 are the compositions of olivine, clinopyroxene, amphibole, garnet, spinel and dolomite that could fractionate from carbonate-rich liquids. Residual liquids could only reach the two-liquid field after appreciable crystallization of dolomite and olivine.

Based on our vapour-undersaturated experiments we conclude that carbonatitic melts are unlikely to be produced by immiscible exsolution from carbonated silicate liquids at pressures between 20 and 30 kbar. The immiscibility field in both sets of experiments is far removed from natural mantle melts. We do not expect the presence of excess vapour to modify this conclusion. High-pressure partial melting and liquid immiscibility at lower pressures, however, remain viable processes for generating carbonatitic melts. We are now measuring how the immiscibility field expands with decreasing pressure. We anticipate that carbonated silicate melts derived at higher pressures may intersect a two-liquid field between 10 and 20 kbar[5-7]. ☐

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Macromolecular resolution of fossilized muscle tissue from an elopomorph fish

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THE Santana Formation (Lower Cretaceous, Aptian/Lower Albian) of Ceara, Brazil, contains many exceptionally preserved fish fossils within carbonate concretions[1,2]. These concretions formed before compaction of the sediment and nucleated around carcasses of fish killed in mass-mortality events. Some concretions may contain several fish, preserved in three dimensions with fully articulated skeletons[1]. A few specimens contain calcium phosphate in the form of cryptocrystalline hydroxyapatite. This occurs as coatings on bones and as a replacement of tissues. Samples of mineralized soft tissues can easily be liberated from the concretions by immersion in 10% acetic acid[3]. Of several mineral phases often preserving soft tissues, calcium phosphate and silica probably offer the greatest resolution of detail[4]. Fossilized soft tissues are most frequently found in fishes, although in the Santana Formation pterosaur wing membrane[5] and the cuticles of arthropods[6,7] have been reported. I report here the study of striated muscle tissue from a fossil elopomorph fish[8] from this formation, in which subcellular ultrastructural features are distinguishable. The exceptional degree of preservation of the specimen raises interesting questions about the mechanism of fossilization.

A specimen of the elopomorph fish[8] *Notelops brama* (Agassiz) in which phosphatization of soft tissues has been extensive, indicates that preservation occurred at the macromolecular level before and during postmortem degradation of the tissues[9]. This resulted in the detailed replacement of structures at the subcellular level in some tissue types. This is most marked in phosphatized striated muscle tissue, where individual muscle fibres are preserved. The muscle fibres are replaced by phosphate in which individual crystallites are less than 100 nm long. Crystallites may be aggregated into microspheres with diameters of 1-2 µm, or as roughly cubic aggregates 0.7-0.8 µm in diameter. In the latter case, preserved muscle fibres show excellent detail of ultrastructure. Here crystallite orientation seems to have been controlled by the orientation of structural proteins, and other biomacromolecules within the fibres. The most obvious result of this is the retention of the sarcomeres in striated muscle fibres (Fig. 1*a*). This fossil banding, equivalent to the I and A bands of modern striated muscle, is highlighted by parallel rows of paired hydroxyapatite crystallite aggregates (Fig. 1*b*). The boundary between crystallites within crystallite pairs may correspond to the H band of individual sarcomeres, whereas the boundary between adjacent crystallite pairs may represent the Z band. Sarcomere length in the fossil material is ~2 µm in compact fibres. Some fibres show that separation between lithified sarcomeres has occurred, but this is most likely a post-lithification artefact. The process of phosphatization took place during active decomposition of the muscle fibres, and individual fibrils can frequently be observed.

Other ultrastructural features of the muscle fibres which have been preserved include the sarcolemma (Fig. 1*c*) and rows of cell nuclei along the muscle fibre surfaces (Fig. 1*d*). In the former case there is sometimes no phosphatization of the enclosed muscle fibre, resulting in a stack of phosphatized sarcolemma membranes. The length of muscle fibres varies according to the size of the fish, and hence to the length of individual myoseptal blocks. Fibre diameter is generally of the order of 50 µm, and preserved sarcolemma is ~1 µm thick. Cell nuclei have been observed on several muscle fibres. The nuclei are ovoid, ~8 µm long and 4 µm in diameter, and arranged

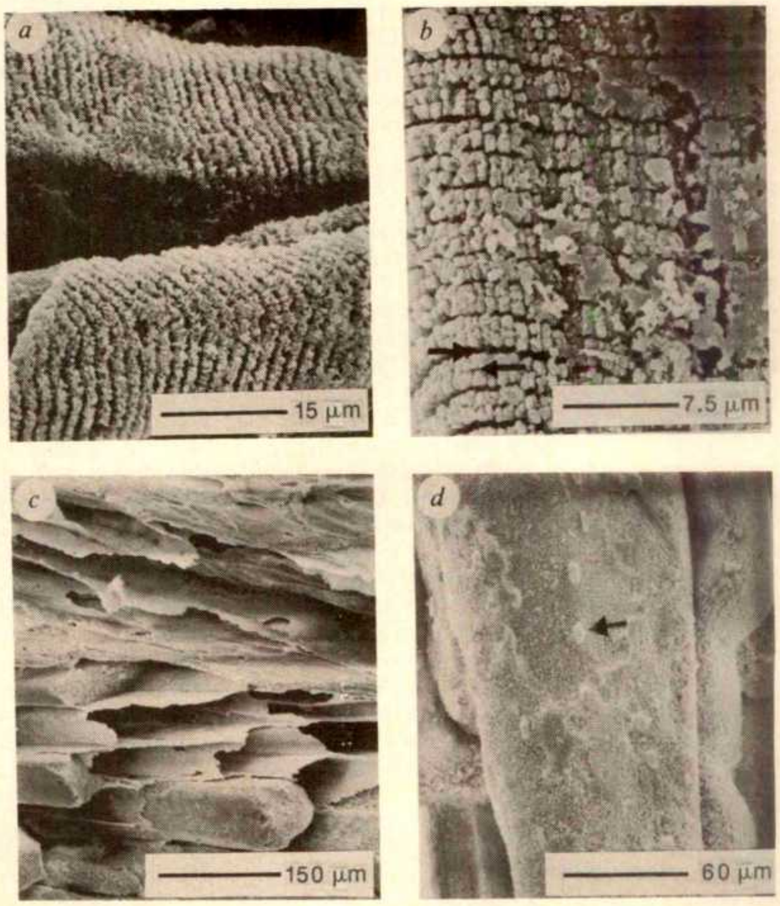

FIG. 1 Scanning electron micrographs of soft tissues from the Cretaceous actinopterygian fish *Notelops brama* (Agassiz), preserved by metasomatic replacement with calcium phosphate and prepared in 10% acetic acid. *a*, Two striated muscle fibres showing well-preserved banding. *b*, Detail of sarcomere preservation showing individual crystallite aggregates of calcium phosphate arranged in pairs; arrows indicate zones interpreted as H and Z bands. *c*, Unusual situation where preservation of sarcolemma has occurred, but preservation of fibres is incomplete. The result is a block of three-dimensionally preserved cell membranes. *d*, Striated muscle fibre showing patches of preserved sarcolemma, sarcomeres and rows of cell nuclei (arrowed).

in rows with long axes parallel to the length of the muscle fibre.

The presence of exceptionally preserved soft tissues raises several questions regarding both the rate and mechanism of fossilization. Many models for exceptional preservation require rapid burial, anoxicity, or both[10-12]. There is evidence for scavenging of many of the exceptionally preserved Santana specimens suggesting that anoxic and rapid burial models are not applicable here. The Santana fossils are associated with laminated organic material, and I suggest that bacterial sealing as proposed by Seilacher *et al.*[11] is a more appropriate model for their exceptional preservation.

Bacterial breakdown of soft tissues might be inhibited by sterilization of bottom waters by high salinity or very low temperatures, thus prolonging their availability for the slow process of fossilization. At a palaeolatitude of 10° S, the Santana Basin was unlikely to have been floored by very cold water, but bottom water of high salinity may have been present[1] as evaporites occur below.

For soft tissues to be so well preserved mineralization must either take place rapidly (in hours rather than days) or bacterial decomposition must be inhibited[12]. Most models regard inhibition of bacterial decomposition as an acceptable mechanism; keeping the soft tissues 'on ice' while fossilization takes place. Gill and muscle degrade rapidly after death; within the first hour such tissues lose their regidity and may display collapse structures. Santana fossil tissues show preservation of these features, suggesting that phosphatization occurred during active decomposition[9].

The mechanism of phosphatization is not fully understood. It may in part be analogous to the biomineralization process in vertebrates. Here, ultramicroscopic crystallites of apatite nucleate within specific sites in collagen or collagen-like proteins[13]. This mechanism is a possibility for the fossil fish because of the abundance of tissues rich in structural proteins. But phosphatizing bacteria have been reported in sediments, where they have an important role in the production of phosphorites[14]. Shapiro[15] demonstrated that environmental fluctuations (changes in temperature and/or oxygen levels) can induce rapid precipitation of phosphate by microorganisms, and a similar bacterially mediated process may be important in the fossilization of soft tissues[1,16]. ☐

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Experimental reversal of courtship roles in an insect

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STUDIES of sexual selection[1] commonly examine female mating preferences or intermale competition[2], but there have been few experimental tests of factors controlling why these, the 'typical courtship roles', exist in the first place. Although the distribution of mates can affect sexual selection, and thus the degree of sexual competition[3], it is the typically lower investment in offspring by males relative to females that is thought to determine the usually greater potential mating frequency, and hence greater sexual competition, among males[4,5]. Reversal in the typical courtship roles is, therefore, expected to occur where there is high male parental investment[4]. The courtship roles are generally considered to be fixed for any particular species because of the usually inflexible nature of adaptations for parental care. Certain katydids (Orthoptera: Tettigoniidae)[6-8], however, show intraspecific variation in these roles. This has been suggested to result from variation in the relative importance of male parental investment[9] in the production of offspring. Male parental investment comprises the nutritional value of the spermotophore eaten by the female. When other food is scarce therefore, females are predicted to compete sexually for males and their offerings[7,8,10]. Here we confirm this prediction and show that an increase in food from a low level results in a change from role-reversal to the typical roles; this supports the theory[4,5] that variation in relative male parental investment controls sexual differences.

Both male and female katydids invest parentally in eggs. The male transfers a meal consisting of the spermatophylax (part of the spermatophore) to his mate, the nutrients of which are translocated to eggs[11] and increase both number and fitness of offspring sired[9,12,13]. For several species, the fitness-enhancing benefits to females of this costly[13-15] nutrient gift have apparently led to sexual selection among females associated with both mate choice by males and inter-female aggression[6-8,16]. In these species, however, this courtship role-reversal is found only in certain populations. Such a role-reversal is argued to be controlled by food availability[6-8] because a scarcity of food: (1) decreases the number of sexually active males and thus their large spermatophylax contributions; and (2) increases the mating (spermatophylax-acquiring) frequency of hungry females[15]. Therefore, with such a decrease in food availability in nature, males rather than females are predicted to be the choosy sex because of the relative scarcity of males that have gained enough nutrients to donate parental investment. Because of the cost of the male contribution[13-15] (ultimately in terms of future offspring sired[5,9]), its enhanced value represents an increase in relative male parental investment[7,10]. An experimental test of the hypothesis that diet controls sexual differences would, therefore, represent a test of the influence of relative parental investment.

The subject is an undescribed genus and species of zaprochiline katydid that feeds only on the pollen of certain spring-flowering plants[17,18]. Large numbers of feeding individuals and sexually signalling (calling) males can be found on a single food plant[18]. In nature the species showed a courtship role-reversal early in the season when feeding from flowers, particularly kangaroo paws (*Anigozanthos manglesii*), that seemed to produce relatively little pollen. Later in the season, when there was an abundance of pollen available on the long flower spikes of *Xanthorrhoea preissei*, fewer females were mating and females became choosier than males[8]. Although this switch in mating system was correlated with an apparent increase in pollen availability, alternative causal explanations, such as a decrease in mating activity by older females, are plausible[8].

In the present experiments, the effects of such variables were controlled by directly manipulating the amount of pollen available; during the flowering of kangaroo paws early in the season, four caged populations of katydids received supplemental food and four served as controls (Table 1).

TABLE 1 Differences between control and supplemental food treatments

		Males		Females				Sexual behaviour		
Treatment	Cage	Mean ($\bar{x}$) No. of calling males per sample (n=11)	Mean mass of spermatophore glands in mg (n)	Mean number of eggs in ovaries (n)	Mean weight of mature eggs per ♀ in mg (n)	Mean number of matings per female (n)	Opportunity for sexual selection on females ($s^2/\bar{x}$) (n)	Fraction of interactions with ♂ choice (n)	Fraction of interactions with ♀ choice (n)	Fraction of interactions with with ♀-♀ competition (n)
Control	1	0.4	8.8 (19)	4.9 (23)	2.3 (6)	1.4 (24)	0.93 (24)	0.38 (13)	0.08 (12)	0.15 (13)
	3	0.7	8.8 (18)	5.5 (21)	2.2 (12)	1.2 (24)	0.94 (24)	0.44 (9)	0.11 (9)	0.22 (9)
	4	0.1	8.8 (20)	5.9 (22)	2.2 (10)	1.4 (24)	1.16 (24)	0.55 (11)	0 (11)	0.27 (11)
	6	0.3	10.2 (20)	5.7 (23)	2.2 (11)	1.3 (24)	1.06 (24)	0.31 (13)	0 (13)	0.30 (13)
Supplemental food	2	8.1	17.0 (21)	12.5 (24)	2.5 (22)	0.6 (25)	0.52 (24)	0 (8)	0.69 (8)	0 (8)
	5	4.7	13.6 (21)	5.7 (19)	2.4 (10)	0.7 (24)	0.55 (24)	0.14 (7)	0.43 (7)	0 (7)
	7	5.7	14.9 (22)	7.5 (20)	2.6 (10)	0.8 (24)	0.49 (24)	0 (5)	0.20 (5)	0 (5)
	8	7.9	18.5 (23)	11.0 (24)	2.5 (20)	0.6 (23)	0.86 (23)	0.06 (8)	0.38 (8)	0 (8)
Difference between treatments (Mann–Whitney test) (P)		0.002	0.002	0.08	0.002	0.002	0.002	0.002	0.002	0.002

The species is catalogued in the Australian National Insect Collection (ANIC) as Gen. Nov. 22, sp. 1 of the Zaprochilinae (D. C. F. Rentz, in preparation). (Voucher specimens were deposited in the ANIC[18].) The study site, also that of previous studies[8,18] is native bush of King's Park, Western Australia[23]. We placed cuboidal fibreglass-screened cages, 0.75 m linear dimensions, over eight clumps of flowering kangaroo paws for 14 days in September 1989. Four cages were randomly selected as supplemental food replicates and into each were placed two artificial *X. preissei* flower spikes produced by coating the distal 15–20 cm of dried sections of flower stalks first with dilute honey, then pollen (bee-collected from Western Australian plants). The remaining four cages were controls receiving only the dried stalks. We placed individually marked zaprochilines, 24 of each sex, into each cage (such densities occur in nature). The behaviour of insects in one replicate of each treatment was observed each evening for a total of 12 sampling days. Observations on focal cages were made from sunset (~18:30) until ~22:00, the period of mating activity[8]. At about 20:30 each day (except one, lost due to rain), we censused the calling males and mated (spermatophore-carrying) females in each of the nonfocal cages. They seemed to behave naturally in the cages; as in nature[8,18], almost all calling males perched on the stems of food plants allowing the observer unobstructed views (using red light) of sexual interactions. At the end of the experiment all insects were collected and later measured and dissected to determine fecundity, weight of (two randomly selected) eggs, and spermatophore gland weight.

Mating in this species begins when a receptive female mounts a male after responding to his ultrasonic call[18]. After coupling the male bends behind the female and the spermatophore is transferred several minutes later. Following separation the female bends and starts to eat the gelatinous spermatophylax which surrounds the ampulla[8,18] (the ampulla transfers sperm into the female). Mate rejection was recorded if the male or female moved away from its partner before spermatophore transfer[8]. Following rejection by a male, females often followed the male and recoupling sometimes occurred. In the case where a female rejected a male, males groped their terminalia in the direction of the retreating female. Only males can reject mates after pairs have coupled genitalia[8]. Sexual competition between females occurs when two females grapple either while responding to the same calling male or after a female disrupts a mating pair. No fighting between males has been noted in this species but it is possible that competition occurs through acoustical displays[8,18].

The results were as predicted. As for unmanipulated field populations[8], there was a reversal in the courtship roles when the only food available to zaprochilines was pollen from kangaroo paws (control); such a reversal was not evident when the katydids received supplemental pollen. Quantitative results are presented in Table 1. To maintain statistical independence, if individuals were represented in more than one interaction only one (randomly determined) interaction was used. Fights between females over males were observed in control cages but never in food-supplemented cages. The frequency of rejection of females by males was significantly higher in the control cages, whereas the rejection of mates by females was most frequent in food-supplemented cages.

Courtship role-reversal in control cages was due to the predicted presence of fewer sexually active males than females; there were significantly fewer calling males in control cages than in food-supplemented cages. The greater proportion of sexually active males in supplemented cages was a result of the increase in the number of males obtaining sufficient pollen to produce spermatophylax donations. This is evidenced by the significantly larger spermatophore-producing glands of food-supplemented males relative to control males. After mating, male katydids call only when the spermatophore glands have been replenished[13] (even when starved, such males do not reduce the size of the subsequent spermatophore[15]). The difference between treatments in the ratio of sexually available females and males is due apparently both to the increase in spermatophore production by food-supplemented males and to the decrease in the mating (spermatophylax-acquiring) frequency of the now food-sated females in this treatment. The number of matings by females in supplemented cages was about half that observed in controls. This accords with observations of female zaprochilines on varying unmanipulated diets in the field[8]. Fecundity and egg size are increased both by additional spermatophylax[13] and apparently by additional pollen[8] eaten, so the higher mating frequency of control females probably acts to increase fecundity. Additional courtship meals in our experiments, however, did not seem completely to compensate for the decreased availability of pollen because control females had lower fecundities and smaller mature eggs compared to food-supplemented females (Table 1).

The opportunity for sexual selection to favour particular females was estimated in a between-treatment comparison of the distributions of variances in female mating success ($s^2/\bar{x}$) (refs 19, 20). As predicted, variances were significantly higher in control than supplemented cages. The body mass of females is expected to be a sexually selected trait as males of this[8] and other katydids[7,16] prefer heavier, more fecund females and larger female zaprochilines usually win fights over pollen[8]. Thus we predicted a mating advantage for heavy females in control but not in food-supplemented cages. This prediction was supported. The regression of body mass on mating frequency was significant for the control (slope = 0.09, $r^2=0.10$, $F_{1,85}=9.6$, $P=0.003$) but not for supplemented cages (slope = −0.01, $r^2=0.01$, $F_{1,78}=0.9$, $P=0.34$) (the four replicates within each treatment were pooled after showing that slopes within treatments were homogeneous ($P>0.05$)). Such a relationship in low cages is, however, predicted not only by the mating success hypothesis but also by the fact that more courtship meals (matings) may increase female mass.

A decrease in pollen available to the zaprochilines causes male parental investment in the nutritious spermatophore to increase in value for offspring production. This change in value does not, however, result in an increase in female choice of males able to supply high quality donations because there is no opportunity for such choice as a result of the large reduction in the proportion of nurturant males. This relative scarcity of males is caused not only by the fact that few males have obtained enough pollen to produce a spermatophore, but also by an increase in mating frequency of females as they seek additional courtship meals[15]. Thus females are subjected to heightened sexual selection associated both with fights between females for access to nurturant males and with mate choice by males. We conclude that the facultative nature of courtship roles in this species results from variation in the relative importance of male parental investment. The results refute alternative explanations for similar behavioural variation in nature[6,8], including the suggestion that such variation may be due to differences in population density[21]. The fact that manipulation of relative male parental investment[10] controls sexual differences also argues against the contention that increased male parental investment results from decreased competition between males[19], rather than the converse as argued by Williams[4] and Trivers[5].

Variation in relative parental investments thus seems to cause greatly differing patterns of sexual selection in brief spans of ecological time. Variation in resource availability is likely to determine the relative importance of the male contribution in any species in which females or offspring receive food from the mate. Such paternal investment occurs in a variety of taxa, and includes the donation of prey in birds, and of prey and a variety of male-produced secretions in insects[22]. Therefore, the facultative nature of the courtship roles is unlikely to be unique to spermatophore-consuming katydids. □

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Cortical microstimulation influences perceptual judgements of motion direction

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NEURONS in the visual cortex respond selectively to perceptually salient features of the visual scene, such as the direction and speed of moving objects, the orientation of local contours, or the colour or relative depth of a visual pattern. It is commonly assumed that the brain constructs its percept of the visual scene from information encoded in the selective responses of such neurons. We have now tested this hypothesis directly by measuring the effect on psychophysical performance of modifying the firing rates of physiologically characterized neurons. We required rhesus monkeys to report the direction of motion in a visual display while we electrically stimulated clusters of directionally selective neurons in the middle temporal visual area (MT, or V5), an extrastriate area that plays a prominent role in the analysis of visual motion information[1–8]. Microstimulation biased the animals' judgements towards the direction of motion encoded by the stimulated neurons. This result indicates that physiological properties measured at the neuronal level can be causally related to a specific aspect of perceptual performance.

Like other cortical sensory areas, MT is organized in a columnar fashion so that clusters of neighbouring neurons have similar physiological properties[9]. In MT, neurons in a single cortical column discharge a burst of action potentials in response to motion in a 'preferred' direction, but yield little or no response to motion in the opposite or 'null' direction. The preferred direction of motion varies systematically from column to column so that MT contains a complete representation of motion direction at each location in the visual field. Consequently, a microstimulation current that selectively elevates the discharge rate of a small cluster of MT neurons should enhance the intracortical signal related to a particular direction of motion. In primate motor cortex, a 10-μA pulse of cathodal current directly activates neurons within ~85 μm of the electrode tip[10]. To activate local clusters of MT neurons, we therefore applied 10 μA stimulating pulses (0.2-msec pulses, 200 Hz, biphasic) to selected sites in which neurons encountered over 150 μm of electrode travel had similar preferred directions. Although we attempted to confine direct excitation to a local cluster, neurons remote from the stimulation site were probably activated trans-synaptically[11]. But activation of remote neurons does not necessarily imply a loss of functional selectivity; there is increasing evidence that cortical columns are preferentially connected with other columns having similar response properties[12,13]. These considerations suggest that microstimulation in our experiments may have activated a circuit of neurons encoding a particular direction of motion.

Our methods for electrophysiological recording and for monitoring eye movements in trained rhesus monkeys were adapted from those of Wurtz and colleagues[14,15], and our psychophysical methods were based on those described by Newsome and Paré[6]. Figure 1 illustrates the procedures used in the present experiments. In brief, three rhesus monkeys were trained to discriminate the direction of motion in a random dot display shown on a video screen. In the display, a specifiable percentage of the dots carried a constant-velocity or 'correlated' motion signal while the remaining dots moved in random directions, creating a masking motion noise. We varied the strength of the motion signal by changing the percentage of dots in correlated motion. For each experiment a new stimulation site was selected and the motion display was placed in the multineuron receptive field mapped at the stimulation site. On a given trial the monkey maintained visual fixation on a stationary point of light while viewing the random dot stimulus for one second. The correlated motion signal was randomly selected to be in either the preferred or null direction of the local cluster of neurons. After the viewing interval, the monkey indicated its judgement of motion direction by making a saccadic eye movement to one of two light-emitting diodes corresponding to the possible directions of the motion stimulus. The monkey received a liquid reward for a correct choice. An experiment consisted of 640 randomly interleaved trials. On half of the trials we applied electrical stimulation that began and ended simultaneously with the onset and offset of the random dot stimulus. The monkeys performed trials at four correlation levels—0% correlation and three levels near psychophysical threshold. The reward contingencies were identical on stimulated and nonstimulated trials.

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If microstimulation enhances the intracortical signal related to the preferred direction of motion of the local cluster of neurons, we would expect stimulation to bias the animal's psychophysical judgements towards that direction. Figure 2 shows the results of two experiments in which microstimulation had such an effect. For both experiments, the proportion of decisions in favour of the preferred direction ('preferred decisions') is plotted against the strength of the motion signal expressed as the percentage of dots in correlated motion. The closed symbols represent trials with electrical stimulation; open symbols correspond to nonstimulated trials. Positive correlations indicate motion in the preferred direction and negative correlations represent motion in the opposite direction. Comparing performance on stimulated and nonstimulated trials, one can see that at every correlation level the monkey made more preferred decisions when electrical stimulation accompanied the visual stimulus, with a net increase of 43 preferred decisions in Fig. 2*a* and 118 preferred decisions in Fig. 2*b*.

In both experiments of Fig. 2, the increase in preferred decisions due to microstimulation can be described as a leftward shift of the psychometric function. The magnitude of the leftward shift quantifies the microstimulation effect in units of the visual stimulus. In other words, the magnitude of the leftward shift, expressed as percentage of correlated dots, corresponds to the visual stimulus change that would mimic the behavioural effect of microstimulation. We employed logistic regression analysis[16] to measure the magnitude and statistical significance of the stimulation-induced shift of the psychometric function. For the experiment of Fig. 2*a*, the effect of microstimulation was behaviourally equivalent to the addition of 7.7% correlated dots to the visual stimulus and was highly significant ($P \leq 0.0001$). For the much larger effect in Fig. 2*b*, the effect of microstimulation was behaviourally equivalent to the addition of 20.1% correlated dots ($P \leq 0.0001$).

Microstimulation caused statistically significant shifts in the psychometric function ($P < 0.05$) in 18 of 38 experiments in one monkey, in 9 of 16 experiments in a second monkey, and in 3 of 8 in a third. Figure 3 shows for each experiment the magnitude of the stimulation-induced shift expressed as percentage of correlated dots. Positive values correspond to leftward shifts in the psychometric function and negative values correspond to rightward shifts. Striped bars indicate experiments in which the shift of the psychometric function was statistically significant. In 29 of the 30 experiments that yielded significant effects, microstimulation shifted the psychometric function leftwards, indicating an increase in preferred decisions. In the remaining experiment, microstimulation caused a highly significant rightward shift, an observation that could be explained if microstimulation had a large effect on nearby columns whose preferred direction was opposite to that of the target column. This is a plausible explanation for the single counterintuitive result as adjacent columns of MT neurons sometimes have opposite preferred directions[9].

The data in Fig. 3 show that microstimulation biased the monkeys' perceptual decisions towards the preferred direction of the stimulated neurons. The result is consistent with the notion that focal microstimulation enhances the sensory representation of one direction of motion relative to others. An alternative

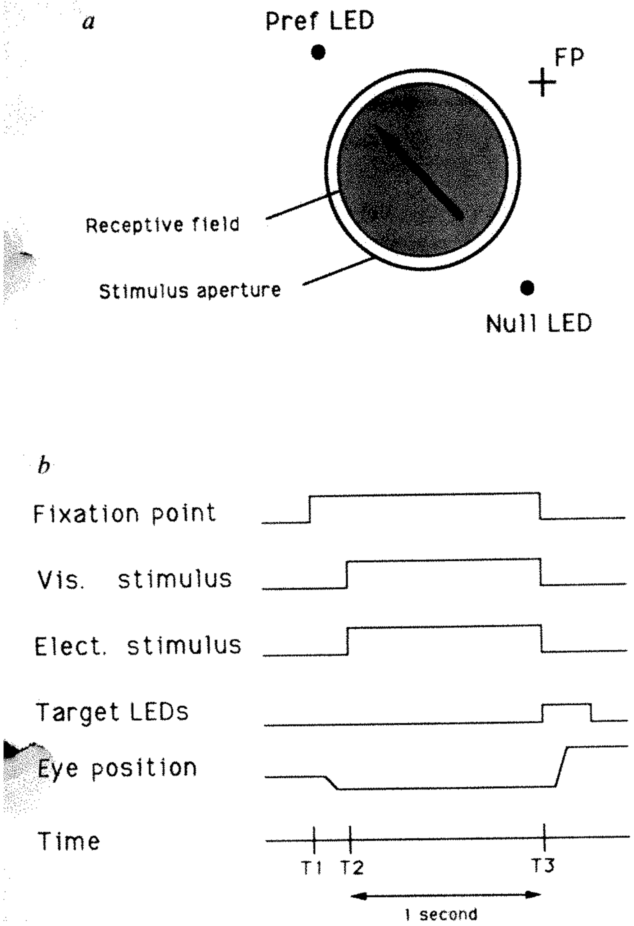

FIG. 1 The experimental protocol used to measure the effect of microstimulation on perceptual judgements of motion direction. *a*, Schematic diagram showing the spatial arrangement of the fixation point (FP), receptive field (shaded), stimulus aperture (thick circle) and response light emitting diodes (LEDs). While the monkey centred its gaze on the fixation point, we mapped the receptive field and identified the preferred direction (arrow) and speed of motion of neurons at the stimulation site. The random dot motion display was presented within a circular aperture whose dimensions and location were matched to those of the multi-neuron receptive field, and the speed of the motion signal was set equal to the preferred speed of the neurons. On a given trial, the motion signal in the visual display was randomly selected to be in the neurons' preferred direction (arrow) or in the null direction (opposite to arrow). The strength of the motion signal was randomly varied among four possible correlation levels: 0% correlation or one of three levels near psychophysical threshold. The monkey viewed the visual display for one second while maintaining its gaze on the fixation point. After the viewing interval, the monkey indicated its choice of motion direction by making a saccadic eye movement to one of two light emitting diodes (Pref LED and Null LED) that corresponded to the two possible directions of motion. Note that in all respects the visual display was tailored so that the demands of the psychophysical task were well matched to the information supplied by the neurons at the stimulation site. Since we tailored the visual display in this manner at each stimulation site, the spatial arrangement of the receptive field, stimulus aperture, preferred–null axis of motion, and response LEDs varied considerably from site to site. *b*, Schematic drawing illustrating the temporal sequence of events during a microstimulation trial. At time T1 the fixation point appeared and the monkey transferred its gaze to the fixation point, as indicated by the deflection in the eye position trace. At time T2 the visual (Vis.) stimulus appeared and the train of electrical (Elect.) stimulation pulses began. The monkey was required to maintain fixation for one second until time T3; if the monkey broke fixation during this interval the trial was aborted. The fixation point, visual stimulus and microstimulation pulses were turned off at time T3, and the target LEDs turned on. The monkey then indicated its judgement of motion direction by making a saccadic eye movement to one of the two response LEDs. A liquid reward was given for a correct choice, and an incorrect choice was penalized with a brief 'time-out' period. Unstimulated trials differed only in that no electrical stimulation accompanied the visual stimulus. The reward contingencies were identical for trials with and without stimulation.

explanation, however, is that microstimulation had a direct effect on the operant response, a saccadic eye movement. The latter hypothesis seems unlikely for several reasons. First, physiological recordings made during saccadic eye movements have failed to find any evidence of motor signals in MT[17]. Second, lesions of MT have no effect on saccades to stationary targets[18]. Third, control experiments show that microstimulation applied during the intertrial interval has no effect on eye movements. Finally, the long latency (>1 s) between the onset of microstimulation and execution of the saccade argues against a direct effect of stimulating current on motor signals. We therefore consider it highly probable that microstimulation affected the sensory signals underlying perceptual judgements of motion direction rather than motor signals related to the saccadic eye movement *per se*.

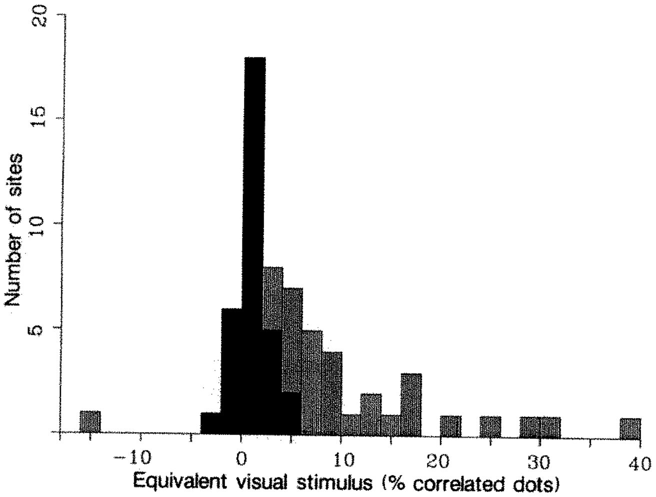

FIG. 2 Effect of microstimulation on psychophysical performance for two stimulation sites in MT. The abscissa indicates the strength of the motion signal in percentage of correlated dots. Positive correlation values indicate motion in the neurons' preferred direction; negative values correspond to motion in the opposite—'null'—direction. The ordinate shows the proportion of trials in which the monkey judged motion to be in the preferred direction of the stimulated neurons (preferred decision). Each data point is based on 40 trials, except for 0% correlation for which 80 trials were conducted. The correlation levels were selected so that the monkey would judge the direction of motion correctly in $\sim$70% of the trials in a block. In half the trials (●) microstimulation was applied simultaneously with the visual stimulus; the other trials (○) contained no microstimulation. For each experiment we employed logistic regression analysis[16] to fit sigmoidal curves of equal slope to the stimulated and nonstimulated data; the curves thus derived provided an acceptable fit to most of the data. *a*, Typical experiment in which stimulation biased the monkey's perceptual decisions towards the preferred direction of the stimulated neurons. The psychometric function for the stimulated trials was shifted leftwards by 7.7% correlated dots. The magnitude of the shift indicates that the behavioural effect of microstimulation could have been reproduced by adding to the visual stimulus 7.7% correlated dots in the preferred direction. *b*, Experiment in which microstimulation induced a leftward shift of 20.1% correlated dots, one of the larger effects we observed. The different appearance of the 'no stimulation' curves in *a* and *b* resulted largely from differences in choice bias in the two experiments. In the no stimulation condition in *a*, the monkey favoured the preferred direction on 56% of the trials at 0% correlation. Note that with no choice bias, the monkey would have made 50% preferred decisions at 0% correlation; in this experiment, therefore, the monkey had a very small choice bias. In the no stimulation condition in *b*, however, the monkey made preferred decisions on only 33% of the trials at 0% correlation. In this experiment, the monkey had a pronounced bias towards the null direction response LED; the effects of this bias are evident at other points on the no stimulation curve as well. Choice bias is a common phenomenon in human and animal psychophysics, and its presence and intensity in any particular experiment is difficult to predict. Most bias values observed in the present experiments were within the range observed in previous experiments that did not involve electrical microstimulation.

As the multi-unit receptive field at a given microstimulation site occupies a small portion of the visual field, it is desirable to know whether the perceptual effects of microstimulation are similarly localized in visual space. To answer this question, we required a monkey to perform the psychophysical task in the usual manner, but applied microstimulation to a topographically noncorresponding site in MT. In these experiments there was no overlap betwen the visual display aperture and the receptive field at the stimulation site. The effect of microstimulation was greatly attenuated or eliminated under these conditions. Thus the microstimulation effects were correlated with the spatial location, as well as the preferred direction, of the receptive field of the stimulated neurons.

Given the complexity of primate visual cortex, which contains more than 20 different visual areas with multiple anatomical interconnections, it is remarkable that local microstimulation of directionally selective neurons can cause a substantial change in psychophysical performance. This result may be less surprising if, as we suggested above, microstimulation trans-synaptically activates an extended circuit of neurons. This amplification of neuronal signals could be accomplished by activation of nearby columns in MT having a similar preferred direction and receptive field location, or by activation of similarly tuned neurons in visual areas other than MT. Although we do not know the full extent of cortex affected by microstimulation, the demonstrated correlation between neuronal preferred direction and psychophysical choice suggests that the activated neurons were functionally related to a particular direction of motion. The data therefore provide evidence causally relating neuronal activity to perceptually judged direction of motion. This experimental approach, which combines electrical microstimulation with multi-neuron physiological analysis and an appropriate perceptual task, may also prove useful for investigating cortical

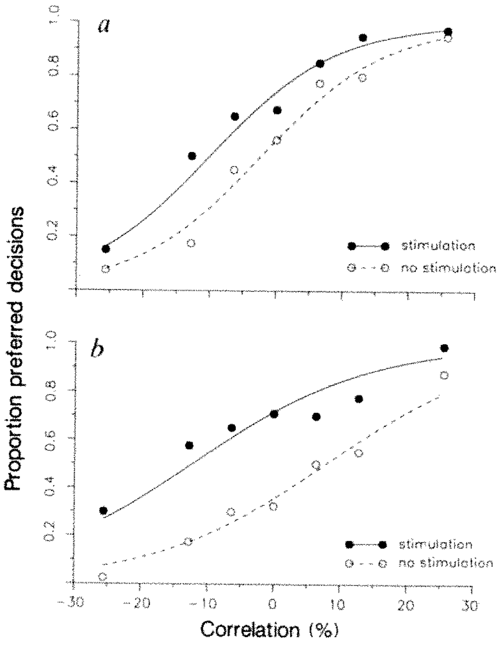

FIG. 3 Frequency histogram showing the magnitude of the stimulation-induced shift in the psychometric function for 62 experiments in three monkeys. The shift in the psychometric function may be thought of as an 'equivalent visual stimulus'—the visual stimulus change that would mimic the behavioural effect of microstimulation. Positive values indicate leftward shifts of the psychometric function; negative values indicate rightward shifts. The striped bars identify experiments in which the effect of microstimulation was statistically significant (logistic regression $P<0.05$). In 29 of the 30 experiments with statistically significant effects, the stimulation-induced shift was leftwards, indicating an increase in decisions favouring the preferred direction of the stimulated neurons.

circuits that contribute to other aspects of visual perception such as orientation, colour and depth. ☐

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Presynaptic enhancement shown by whole-cell recordings of long-term potentiation in hippocampal slices

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LONG-TERM potentiation (LTP) of synaptic transmission in the hippocampus is a widely studied model system for understanding the cellular mechanisms of memory[1–6]. In region CA1, LTP is triggered postsynaptically[7–10] by Ca^{2+}-dependent[11,12] activation of protein kinases[13,14], but the locus of persistent modification remains controversial[15–25]. Statistical analysis of synaptic variability has been proposed as a means of settling this debate[3,26], although a major obstacle has been the poor signal-to-noise ratio of conventional intracellular recordings. We have applied the whole-cell voltage clamp technique[27] to study synaptic transmission in conventional hippocampal slices (compare refs 28–30). Here we report that robust LTP can be recorded with much improved signal resolution and biochemical access to the postsynaptic cell. Prolonged dialysis of the postsynaptic cell blocks the triggering of LTP, with no effect on expression of LTP. The improved signal resolution unmasks a large trial-to-trial variability, reflecting the probabilistic nature of transmitter release[31–35]. Changes in the synaptic variability, and a decrease in the proportion of synaptic failures during LTP, suggest that transmitter release is significantly enhanced.

We made whole-cell voltage clamp recordings[27–30] from CA1 neurons in rat hippocampal slices (400–500 μm). Positive pressure was applied to the recording pipette during penetration of the slice; high-resistance (1-5 GΩ) seals on cell bodies 2-3 cell diameters below the surface were then obtained by suction. After breaking into the cell, synaptic currents were elicited with bipolar stimulation electrodes placed in the stratum radiatum of CA1. Stable synaptic recordings could be maintained for as long as 10 hours.

To optimize the chances of obtaining LTP under whole-cell recording conditions, we used internal solutions with minimal Ca^{2+} buffering to avoid block of LTP[11,12], and with Cs^{+} as the main cation to enhance voltage control of the postsynaptic membrane. We also included ATP and GTP in the internal solution to allow activity of protein kinases or GTP-binding proteins. After a stable baseline period of 10–15 min, we were able to induce LTP by pairing[7,9,10] a steady postsynaptic depolarization to ~0 mV with continued activation of the test pathway (40 stimuli at 2 Hz). This pairing procedure resulted in LTP in 14 out of 18 experiments (Figs 1*c*, 3*a* and 4*c*). By contrast, no potentiation was found in transmission through a simultaneously monitored control pathway that did not receive presynaptic stimuli during the postsynaptic depolarization (Figs 1*d*, 3*b*). Furthermore, synaptic stimulation at 2 Hz without postsynaptic depolarization did not give synaptic enhancement ($n = 5$). Thus, the potentiation was synapse-specific and required a combination of presynaptic activity and postsynaptic depolarization, just as seen in conventional recordings of LTP[1–6,36].

To investigate whether diffusible cytoplasmic factors are involved in potentiation, we attempted to trigger LTP at different times after gaining whole-cell access (Fig. 1*c*, *d*). Pairing soon after starting whole-cell recording (~20 min or less) consistently resulted in LTP lasting >1 hour (Figs 1, 3 and 4). But no potentiation was found with pairing >30 min after whole-cell access (6 out of 7 experiments). One possibility is that some diffusible postsynaptic substance is needed to trigger LTP, but not to maintain the potentiation.

The low background noise of whole-cell recordings allowed us to study small responses evoked with minimal stimulation, only slightly stronger than the highest stimulus that gave all failures. With minimal stimulation, activation is thought to be restricted to a single synapse onto the monitored neuron[25,37]. The resulting synaptic currents showed a large variability between trials that was much greater than the background noise, but their individual time courses were similar (Fig. 1*a*, *b*), supporting the view that they originated from the same synapse[25,37]. There were occasional clear failures (Figs 1*a* and 4*a*, *b*), and sporadic spontaneous events that resembled the elicited response, ranging in amplitude from <1 pA to ~10 pA (Fig. 1*b*, inset).

To understand the basis of the synaptic variability and its possible relation to fluctuations in transmitter release, we modified presynaptic or postsynaptic functions by changing the bathing medium (Fig. 2). Raising the extracellular calcium concentration ($[\mathrm{Ca}^{2+}]_0$) to increase presynaptic release[38] enhanced the synaptic currents (Fig. 2*a*), and changed their amplitude histogram from a highly skewed distribution (Fig. 2*b*) to a nearly symmetrical bell-shape (Fig. 2*c*). By contrast, when we added low concentrations of the glutamate receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione[39] (CNQX) to modify postsynaptic responsiveness, the average synaptic current was dramatically reduced, but the shape of the distribution remained unchanged (Fig. 2*a*, *e*). The distributions before and after CNQX therefore matched closely when normalized by their means (Fig. 2*d*). We obtained similar results with CNQX at lower $[\mathrm{Ca}^{2+}]_0$ (data not shown).

To derive a simple and revealing index of synaptic variability, we computed $V^{-2} = M^2/\sigma^2$, where V is the coefficient of variation, M is the mean synaptic current and σ^2 is the variance about M, for a given epoch of consecutive responses from t to $t+\tau$. We can compute the expected behaviour of M^2/σ^2 if we make assumptions about the mechanisms underlying synaptic transmission. In the simplified case of a binomial distribution of transmitter release, $M^2/\sigma^2 = (Npvz)^2/Np(1-p)(vz)^2 = Np/(1-p)$, where p is the probability of release for each of N available quanta, v is the vesicular content, and z is the postsynaptic response to a fixed amount of transmitter. Thus, M^2/σ^2 is independent of changes in z and is a useful measure of some changes in presynaptic function, but not all factors (for example,

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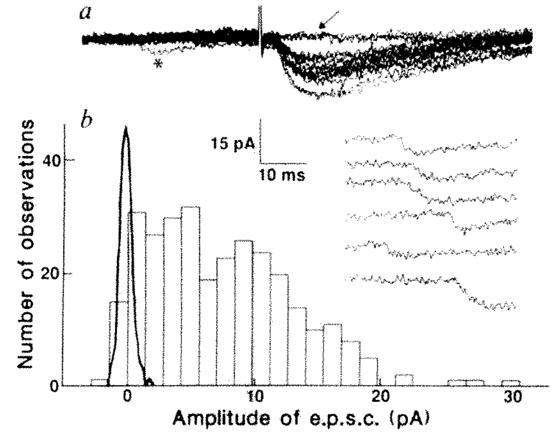
v). It increases in proportion to N, and at least linearly with p, as experimentally confirmed at the neuromuscular junction[31]. The lack of dependence of M^2/σ^2 on the postsynaptic responsiveness (z) holds for more general cases as long as z does not vary from trial to trial within a given epoch (see Fig. 2 legend).

Assuming z is constant for an epoch, the theory predicts that changes in M^2/σ^2 between epochs will result from changes in release characteristics but not postsynaptic modifications. This was tested by examining changes in M^2/σ^2 following interventions known to affect presynaptic or postsynaptic mechanisms. As Fig. 2e illustrates, raising extracellular calcium dramatically increased M^2/σ^2. This was found for all experimental manoeuvres expected to affect presynaptic release: lowering magnesium, application of 4-aminopyridine (data not shown) or increasing the stimulus strength to recruit more afferent fibres (Fig. 2f; $n = 10$). In general, M^2/σ^2 increased at least as much as the mean synaptic current. By contrast, addition of CNQX produced no significant change in M^2/σ^2, despite its large effect on synaptic transmission ($n = 5$; see Fig. 2e, g).

Does M^2/σ^2 change with LTP? Figure 3b compares M^2/σ^2 of the test pathway with that of the control (unpaired) pathway, for the 14 experiments showing potentiation of mean synaptic current (Fig. 3a). Following pairing, M^2/σ^2 increased significantly in the test pathway, with no change in the control pathway. The increase in M^2/σ^2 with LTP could arise from various presynaptic mechanisms: an increase in the number o available vesicles; an increase in the probability of release o some or all vesicles; a non-uniform increase in the amount o transmitter in the vesicles; or changes in non-vesicular release It would not be expected from uniform changes in the number sensitivity or conductance of glutamate receptor channels, or if the effectiveness of charge transfer from spines to dendrites.

If the expression of LTP involves an increased probability o release or a greater number of available quanta, one would expect fewer failures of transmission during LTP. Figure 4 illustrates an experiment where failures and responses were clearly resolvable. The amplitude distribution histogram had a distinct peak at zero amplitude which matched the amplitude distribution of the noise (inset). The area under the 0 pA peak was used to estimate the percentage of failures. In this experiment, the proportion of failures decreased from 27% in control to 11% during LTP. This decrease was a consistent finding in each of five experiments where failures could be clearly resolved ($n = 3$ with minimal stimulation and $n = 2$ with intracellular microelectrode stimulation of a CA3 neuron). The collected data from all five experiments showed a mean (±s.e.m.) decrease in failures from 63 ± 11% in control, to 17 ± 8% after pairing consistent with a greater likelihood of transmitter release during



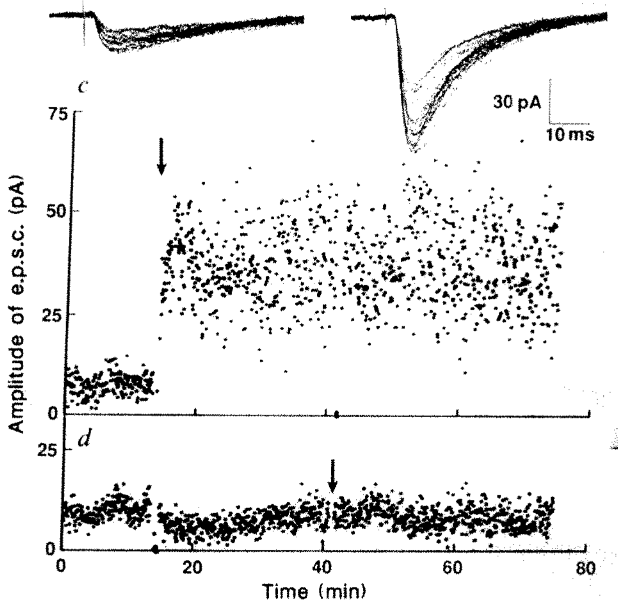


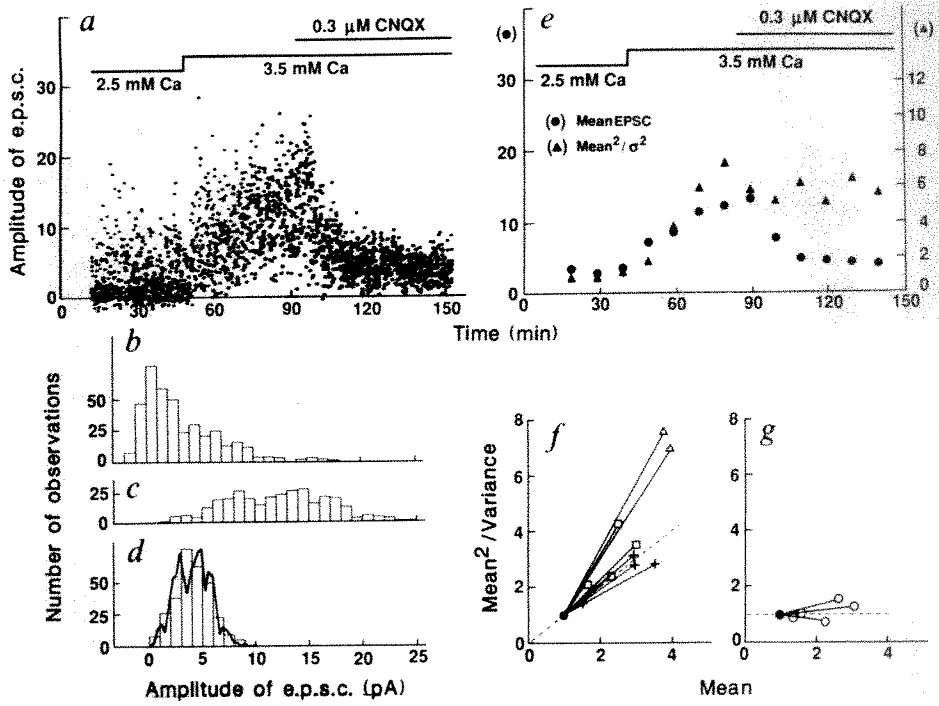
FIG. 1 Whole-cell recordings of synaptic transmission in conventional hippocampal slices show large inter-trial variability, synapse-specific LTP, and loss of induction but not expression, with prolonged dialysis. a, Sixteen consecutive records of excitatory postsynaptic currents (e.p.s.cs), superimposed. Note spontaneous event in baseline period (asterisk) and synaptic failures (arrow). b, Amplitude distribution histograms for 200 consecutive synaptic currents (bars) and baseline noise (smooth curve). Bin sizes were 1.4 pA for e.p.s.c., and 0.14 pA for baseline noise. Inset: Representative spontaneous synaptic currents. Note the variability in amplitude and time course but an otherwise general similarity to evoked responses. c, d, Plots of synaptic responses against time in a postsynaptic cell receiving two independent inputs. c, LTP was selectively induced in one pathway by a pairing procedure 15 min after break-in (arrow); in the other pathway (d), the pairing procedure was applied 25 min later, but failed to induce LTP, in contrast to the maintenance of LTP in c. In the pairing procedure, the cell was depolarized from −70 mV to ~0 mV, while the paired (conditioned) pathway was stimulated 40 times at 2 Hz with no change in stimulus strength. Non-conditioned pathway received no stimuli during the postsynaptic depolarization. Inset: Families of consecutive current records showing e.p.s.cs of conditioned pathway, collected before (left) and 30 min after pairing (right).

METHODS. Transverse hippocampal slices (400–500 μm), obtained from 3–5-week-old rats by standard methods[42], were submerged and superfused continuously with a modified Earle's solution containing (in mM): NaCl, 119; KCl, 2.5; $MgCl_2$, 1.3; $CaCl_2$, 2.5; NaH_2PO_4, 1.0; $NaHCO_3$, 26.2; glucose, 11; picrotoxin, 0.02, and gassed with 95% O_2/5% CO_2 (pH 7.4) at room temperature (22 °C). A cut was made between regions CA3 and CA1 to prevent epileptiform activity. Synaptic transmission was elicited with bipolar stainless steel stimulating electrodes at two locations; each electrode delivered a stimulus every 4 s; stimuli from the two electrodes were separated by 2 s. E.p.s.cs were recorded with a patch electrode (3–7 MΩ tip resistance, no fire polishing or Sylgard coating) in the whole-cell mode (Axopatch 1D). Pipette solution contained (in mM): Caesium gluconate, 100; EGTA, 0.6; $MgCl_2$, 5; ATP, 2; GTP, 0.3; HEPES, 40 (pH 7.2 with CsOH). Holding potential was kept constant at a level between −60 and −70 mV. E.p.s.cs were amplified 50–500 fold, filtered at 1 kHz, digitized at 10 kHz and stored. For a given experiment, e.p.s.c. amplitudes were determined by averaging the current over a 10–20 ms window at the peak response and subtracting a baseline estimate from the same record. A similar procedure was used to measure the background noise before the synaptic stimulation[34]. In a representative series of 17 experiments, the average synaptic current for minimal stimulation was 6.7 pA, in reasonable agreement with estimates of synaptic current from minimal e.p.s.ps recorded with high-resistance microelectrodes[25,34], after allowing for input resistance. Note, however, that the background noise in whole-cell recordings was considerably lower than in the most favourable microelectrode recordings[25,34]. The input resistance in whole-cell recordings was typically 100–200 MΩ.

TP. This conclusion is supported by the overall amplitude istribution, which changes from a skewed shape in control Fig. 4*d*) to a bell-shape during LTP (Fig. 4*e*), as with increasing xtracellular calcium (Fig. 2*b*, *c*).

One of our main findings is that whole-cell recordings can be sed to study synaptic transmission over many hours in neurons that are well below the surface of conventional brain slices (see also ref. 29). This approach is an alternative to methods that require cleaning of the surface of slices[30], and it allows access to deeper cells that are more likely to retain intact dendritic structures and functional properties.

Our experiments show that synapses can display LTP even



IG. 2 Agents acting through presynaptic nechanisms affect shape of distribution istograms and M^2/σ^2, whereas inhibition f postsynaptic receptors does not. *a*, .p.s.c. amplitude plotted against time. athing calcium was raised from 2.5 mM o 3.5 mM, and 0.3 μM CNQX was applied vhere shown. *b*–*d*, Amplitude distribution istograms for 300 e.p.s.cs before nanipulation (*b*), after elevation of calcium *c*), and after subsequent addition of CNQX *d*). Continuous line in *d* shows data from *c*, normalized to mean e.p.s.c. amplitude in *d*. Note close agreement of normalized histograms. *e*, Plot of mean e.p.s.c. amplitude, *M* (circles) and M^2/σ^2 (triangles) for the experiment shown in *a*, with $\tau=10$ min (150 trials). *f*, Plot of M^2/σ^2 against *M* for several presynaptic manipulations: concentration of calcium was increased from 2.5 to 3.5 mM (triangles); magnesium was decreased from 1.3 to 0.65 mM in the presence of 50 μM 5-aminophosphonovaleric acid (squares); increased stimulus strength to recruit more fibres (cross). *g*, Plot of M^2/σ^2 against *M* for experiments in which CNQX (0.2–0.3 μM) was applied to the bath. For each experiment in *f* and *g*, lines connect points obtained before and after manipulation; *M* and M^2/σ^2 were computed for 300 trials before the manipulation and for 300 trials at the peak of the effect, and normalized by the values corresponding to the smaller *M* for that experiment. Note that presynaptic manipulations thought to increase *p* affect M^2/σ^2 more than the mean, whereas increasing stimulus strength, which increases *N*, affects M^2/σ^2 as much as *M*. These findings are expected for a binomial release process (see text). Postsynaptic receptor blockade generally had no affect on M^2/σ^2. This conforms with predictions of a treatment of synaptic responses that does not presuppose binomial statistics, uniformly sized packets, or even vesicular release. In the general case, the release process is considered to behave as a probability density function $R(x)$, where x is allowed to vary over possible values of transmitter release. The postsynaptic response is assumed to be zx, where z is a constant postsynaptic response factor. From the elementary probability theory, the mean synaptic response is given by $\langle zx\rangle$ and the variance about the mean is $\langle(zx-\langle zx\rangle)^2\rangle$, where $\langle\ \rangle$ denotes the expectation for trials within the epoch τ. Then $M^2/\sigma^2=\langle zx\rangle^2/\langle(zx-\langle zx\rangle)^2\rangle=z^2\langle x\rangle^2/(\langle(zx)^2\rangle-\langle zx\rangle^2)=z^2\langle x\rangle^2/z^2(\langle x^2\rangle-\langle x\rangle^2)=\langle x\rangle^2/(\langle x^2\rangle-\langle x\rangle^2)$. Thus M^2/σ^2 depends only on characteristics of the release process for that epoch, and not on z. This formalism assumes that postsynaptic responsivity is linear and does not vary from trial to trial (that is, z is constant) within the epoch τ, as found at the neuromuscular junction[31,32]. It ignores the possibility of rapid and concerted changes in the receptivity of ensembles of receptors or a spatial heterogeneity in receptivity that responds differentially to independent sites of transmitter release.

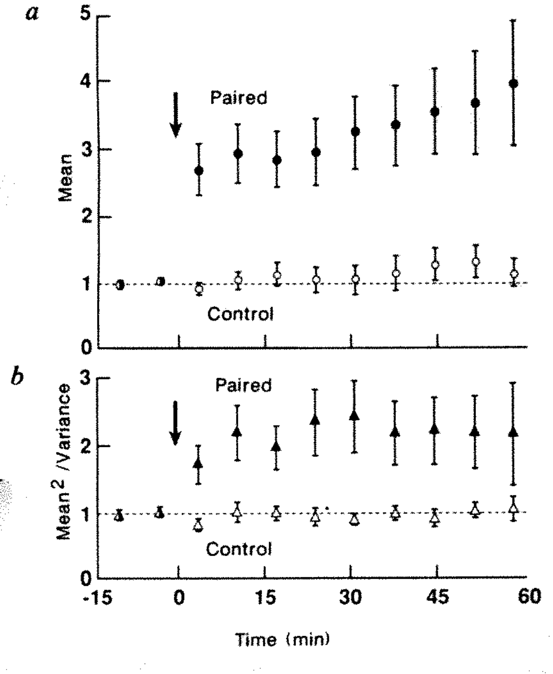


FIG. 3 A synapse-specific increase in M^2/σ^2 associated with LTP. Results from the 14 of 18 experiments that showed LTP with pairing (in the other four experiments with no LTP, M^2/σ^2 remained unchanged; data not shown). *a*, Ensemble averages and s.e.ms of *M* for the paired pathway (filled circles) and the control pathway (open circles, $n=10$; in 4 experiments this pathway was not monitored). Arrow marks time of pairing procedure (as in Figs 1, 2). *b*, Ensemble averages of M^2/σ^2 for paired (filled triangles) and non-paired (open triangles) pathways for the same experiments as in *a*. Note that *M* increased more than M^2/σ^2, particularly 30–60 min after pairing; the late change in *M* may reflect a delayed increase in postsynaptic responsivity[24].

after the postsynaptic cell has been accessed by a whole-cell recording pipette. This opens up the possibility of probing the postsynaptic mechanisms of LTP with a wide range of biochemical compounds, such as large proteins or nucleic acids, that could not be reliably delivered with intracellular microelectrodes. It is interesting that the ability to undergo LTP was lost after longer periods of whole-cell recording, as if some key cytoplasmic constituent were washed out, even though LTP established earlier during the recording did not decay. This contrast supports the idea that induction and persistence of LTP involve different molecular events, possibly in different locations[13,40,41].

Another advantage of whole-cell recordings is that they give much better signal-to-noise than conventional intracellular recordings, facilitating statistical analysis of small synaptic signals. Experiments conform with theory in supporting M^2/σ^2 as a simple measure of changes in presynaptic function. In principle, this approach could be useful in characterizing neuroactive drugs whose locus of action is unknown.

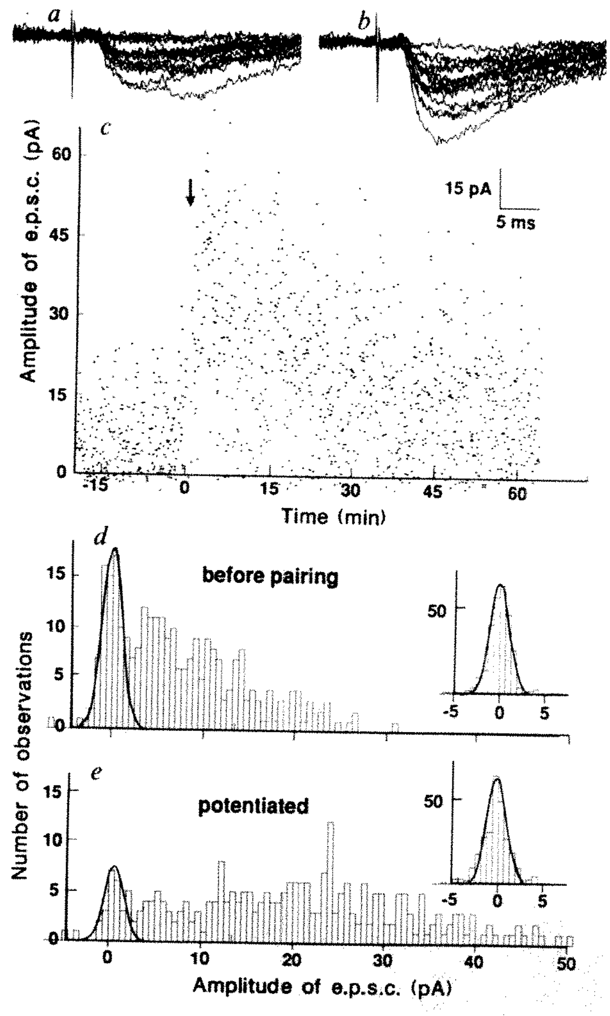


FIG. 4 Decreases in the proportion of synaptic failures associated with LTP. *a*, *b*, Groups of 16 consecutive e.p.s.cs taken before (*a*) and 40 min after (*b*) a pairing procedure to induce LTP. *c*, Amplitude of e.p.s.cs elicited by minimal stimulation plotted against time. Arrow marks pairing procedure (see Fig. 1). *d*, *e*, Amplitude distribution histograms for 17 min epochs before pairing (*d*, from $t=-17$ to $t=0$) and after pairing (*e*, from $t=5$ to $t=22$ min). Smooth curves are gaussians whose height was adjusted to fit the first peak in the amplitude distribution. The half-width of the gaussians, 2.5 pA, was obtained by fitting amplitude histograms of the baseline noise during the same epochs (insets). The proportion of failures, estimated as the area under the gaussian curve, was 27% before pairing (*d*), and 11% between 5 and 22 min after pairing (*e*). Note the change in shape of amplitude distribution histogram in association with 2.8-fold increase in mean synaptic current from *d* to *e*. During a later epoch (from $t=40$ to $t=57$ min, histogram not shown), the synaptic enhancement was 2.1-fold relative to control, and the proportion of failures was 15%.

Our results provide electrophysiological evidence for changes in presynaptic function during LTP, as already suggested[13,15-19]. This conclusion is based on two independent but complementary approaches. The analysis of M^2/σ^2 is relatively model-independent, and does not require failures. M^2/σ^2 increased significantly and remained elevated during LTP; it was unchanged in pathways not undergoing potentiation (Fig. 3*b*). An analysis based on failures gives a more direct view of presynaptic function, but requires unitary events that are large relative to the background noise. In all cases where failures were clearly resolved, their occurrence was greatly diminished during LTP (see Fig. 4*e* for example). Thus, both sets of results support an increase in the likelihood of transmitter release.

Our results do not exclude some change in postsynaptic responsivity (Fig. 3 legend). This possibility[20-22] is supported by a recent report of minimal excitatory postsynaptic potentials (e.p.s.ps) in which no clear change in quantal content was detected in association with a 30% potentiation[25]. The enhancement of transmitter release may have been more pronounced in our experiments because the potentiation was considerably larger (~200%).

Our evidence supporting presynptic changes with LTP is striking in light of previous studies showing that induction is postsynaptic[7-13]. The combination of results makes it difficult to escape the conclusion that a retrograde message must travel from the conditioned postsynaptic cell to modify presynaptic function[1-5]. As the proportion of failures decreases and M^2/σ^2 increases soon after pairing, the retrograde communication must occur promptly. ☐

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Role of abortive retroviral infection of neurons in spongiform CNS degeneration

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RETROVIRUSES are involved in several human neurological diseases with varying pathological features[1–3]. Whether these diseases are due to a direct effect of the virus on nervous system cells is unknown. To gain insight into the pathogenesis of one retroviral neurological disease, we are studying the murine neurotropic retrovirus, Cas-Br-E, which causes lower motor neuron disease associated with spongiform degenerative changes in brain and spinal cord[4]. Central nervous system (CNS) injury seems to be due to direct viral action[5], but the precise target cells of the virus are uncertain. After blood-borne virus enters the CNS it is found in capillary endothelial cells[5–7]. No microscopic evidence for virus within glia or neurons has been found in some studies[6], whereas virus or incomplete particles have been observed in CNS cells in other studies[5,8]. Here we identify the neuron as a major target for Cas-Br-E in the CNS, suggesting that this disease may be a direct result of viral infection of neurons. We also show that envelope protein (Env, encoded by the *env* gene), a major determinant of neurovirulence[9,10], cannot be detected in neurons but is present in non-neuronal cells, although spliced *env* messenger RNA is synthesized in CNS tissue. This suggests that a post-transcriptional step in Cas-Br-E Env protein synthesis is impaired and that the neurological disease may be a consequence of abortive replication of virus in neurons. This may explain the failure to find neuronal infection in other neurological diseases by conventional methods of virus detection.

To determine the target cells for Cas-Br-E in the CNS, we compared the virus–host cell interactions of neurovirulent Cas-Br-E with that of non-neurovirulent Moloney murine leukaemia virus (MoMuLV). Whereas postnatal infection of mice with MoMuLV results in viral infection of primarily haematopoetic cells, mid-gestation infection of embryos allows MoMuLV to infect a wide variety of cells[11], including most cell types in the nervous system. Mice expressing even large amounts of virus in the nervous system do not, however, develop neurological disease. Mid-gestation infection with Cas-Br-E produces a disease that is clinically and pathologically the same as neonatal infection, except that its course is markedly shortened[12].

We infected mice intraperitoneally (i.p.) at birth or at mid-gestation. Spongiform degeneration and marked gliosis were observed in the spinal cord, brain stem, cerebellum and subcortical regions, as seen previously[4]. Cells identified morphologically as large motor neurons were stained when spinal cord sections of mice infected with Cas-Br-E i.p. at birth or at mid-gestation were treated with an anti-MoMuLV antibody that detected Cas-Br-E and MoMuLV viral antigens (Fig. 1). Other cells not readily identified under the light microscope also

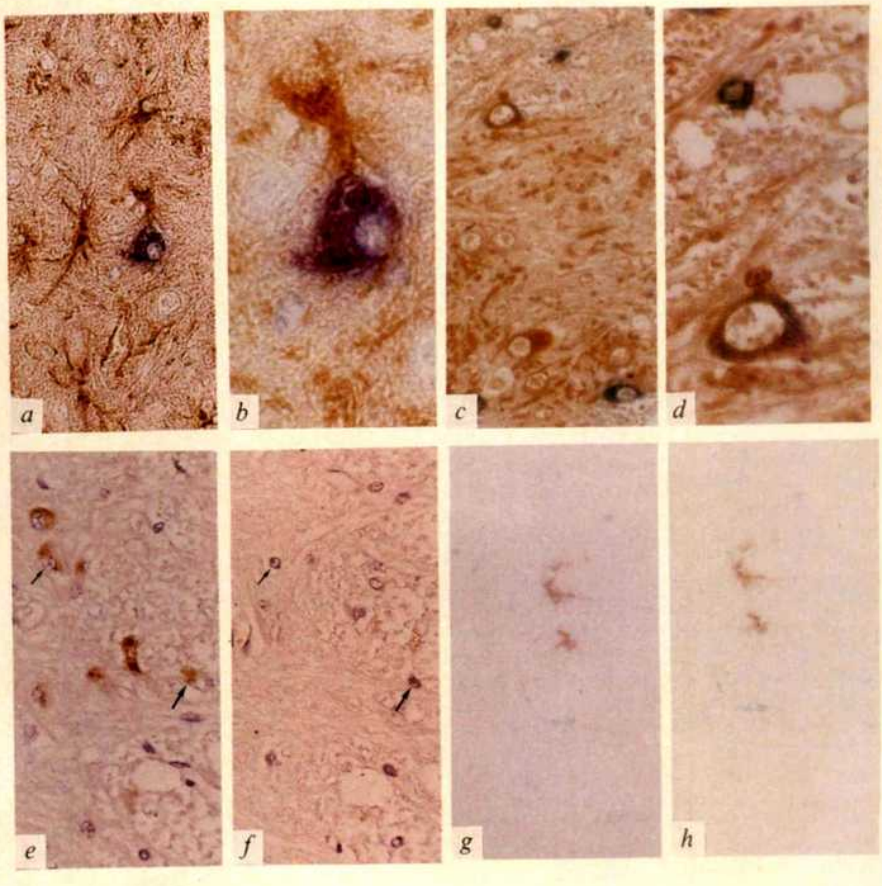

FIG. 1 *a–d*, Double immunohistochemical staining of infected spinal cord sections. Note the colocalization of viral antigens and neurofilament antigens to the same cells. Brown, immunoperoxidase (IMP) staining of glial cells using antibodies to GFAP (*a*, *b*) or neurons using antibodies to neurofilaments (*c*, *d*); purple, alkaline phosphatase (AP) staining of virus-infected cells. *e–h*, Cells visualized by the anti-total virus antiserum were not stained with a polyclonal antibody recognizing gp70 in adjacent sections of Cas-Br-E infected spinal cord. Note lack of reactivity of anti-Env antibody (*f*) as compared with anti-total virus antibody (*e*). Arrows identify identical cells in the adjacent sections. These two antisera gave similar results in MoMuLV-infected tissues (*g*, anti-total virus; *h*, anti-Env). Brown, immunoperoxidase staining of virus-infected cells. Magnification: ×560, *a*, *c*, *e–h*; ×1,400, *b*, *d*. Counterstain in (*e*) and (*f*) is haematoxylin and in *g* and *h* is methyl green.

METHODS. SWR/J mice were infected i.p. as neonates with 0.05 ml virus with a titre of 10^6 XC plaque-forming units per ml or at mid-gestation[7] with approximately 0.05 μl virus of the same titre. Virus was prepared from cells producing cloned MoMuLV or Cas-Br-E (clone NE-8)[17]. Mice infected i.p. with Cas-Br-E were examined from 2 weeks to 5 months post-infection. Mice infected with Cas-Br-E or MoMuLV as mid-gestation embryos were examined at embryonic day 16, postnatal days 14 and 24 and at 2 and 3 months after birth (MoMuLV-infected mice). Four mice were examined at each time point. Mice were anaesthetized with sodium pentabarbital and perfused through the heart, first with 0.9% NaCl containing 100 units heparin per ml (Sigma) and then with Carnoy's fixative, both pre-chilled to 4 °C. Small portions of tissue were then post-fixed in Carnoy's at 4 °C overnight, dehydrated in graded alcohols and xylenes and embedded in paraplast plus. Serial 3-μm sections were cut and heat-baked onto glass slides pretreated with Elmer's glue (Borden). Immunohistochemistry was performed as described[18]. For recognition of viral antigens, slides were incubated with goat antibodies to total virus (NIH 81S107) or to the viral envelope (gift of W. Schäfer) followed by a donkey anti-goat AP conjugate (1:100, Jackson Immunoresearch). Antibody localization was visualized by incubating sections with 1 mg ml^{-1} Fast Blue BB (Sigma), 0.2 mg ml^{-1} napthol AS-MX phosphate in 0.1 M Tris buffer, pH 8.2. For detection of GFAP, sections were incubated with rabbit anti-GFAP antibody (1:800, Accurate), followed by a donkey anti-rabbit IMP conjugate (1:100, Jackson Immunoresearch). For detection of neurofilaments, the avidin-biotin system used mouse anti-neurofilament antibodies (SM32, 1:4000, Sternberger-Meyer) followed by a mouse Vectastain ABC kit (Vector). Antibody localization was visualized using a peroxidase reaction with 3,3′-diaminobenzidine tetrahydrochloride (Aldrich) as the chromagen (1.5 mg ml^{-1}, with 0.1 ml of 3% hydrogen peroxide). Slides were counterstained with methyl green or haematoxylin.

TABLE 1 Cas-Br-E and MoMuLV interactions with cells in the CNS

	Cas-Br-E		MoMuLV	
	Disease	Target cells	Disease	Target cells
Mid-gestation	Paralysis (15–30 days)	Neuron (endothelial, rarely astrocytes)	Leukaemia (3–5 months)	Neuron, glia endothelial
Postnatal	Paralysis (4–6 months)	Neuron (endothelial, rarely astrocytes)	Leukaemia (5–10 months)	None

contained viral antigens. Using antisera against glial fibrillary antigen protein (GFAP) to identify astrocytes, and antisera against neurofilament (NF) subunits to identify neurons, double immunohistochemical staining studies revealed that the vast majority of infected cells were neurons (Fig. 1*a–d*). Infection of astrocytes occurred only rarely, and infection of endothelial cells was seen primarily early after virus exposure. Only a few per cent (at most five) of the infected cells were non-neuronal and non-endothelial. These cells may be oligodendroglia or microglia. Mice infected as mid-gestation embryos with MoMuLV had infected endothelial cells, astrocytes and neurons (Table 1).

Because previous genetic studies had suggested the importance of the *env* gene in the pathogenesis of Cas-Br-E neurological disease[10], tissue sections were also examined with polyclonal antibody reactive against MoMuLV or Cas-Br-E gp70. Unexpectedly, a comparison of immunocytochemical staining of adjacent spinal cord sections prepared from infected Cas-Br-E mice with the anti-Env and anti-total virus sera revealed that the two antisera gave dramatically different results. Neurons visualized by the anti-total virus antibody were not stained in adjacent sections reacted with anti-Env antibody (Fig. 1*e*, *f*), whereas sections of Cas-Br-E-infected spleen and thymus stained similarly with both antisera. Neurons, glia and endothelial cells in mice infected at mid-gestation with MoMuLV were stained with both antisera, as would be expected for a productive virus infection (Fig. 1g, *h*).

To investigate Env protein synthesis in the CNS, western blo analysis was carried out using tissue extracts prepared fron mice infected with Cas-Br-E or MoMuLV and control, uninfected SWR mice. Confirming the results in Fig. 1, no Env protei was detected in the CNS of mice infected with Cas-Br-E, i contrast to mice infected with MoMuLV. (Fig. 2). Viral protein other than Env were present in tissue extracts of Cas-Br-E infected cerebrum, cerebellum, medulla and spinal cord. Th spleen and thymus of Cas-Br-E-infected mice contained all th viral proteins including Env. As expected, all viral protein (including Env) were observed in tissues prepared from mic infected with MoMuLV at mid-gestation and probed with th same antiserum (Fig. 2). An independently prepared polyclona antiserum to Env protein, reactive against both Cas-Br-E an MoMuLV gp70, gave identical results (data not shown). If th Env protein had been present, it should have been detectable as the levels of other viral proteins were comparable in thymu and CNS tissue extracts.

To determine whether the *env* mRNA was present in brai and spinal cord, we examined viral transcription in infecte tissues. Cas-Br-E *env* mRNA was present in CNS tissues (Fig 3) and quantitative analysis of the amount of *env* mRNA i brain and spinal cord compared with other organs revealed n major differences. Thus, a post-transcriptional step in Env synthesis is responsible for aberrant synthesis of Env protein in th CNS.

Our studies identify the neuron as a major target for Cas-Br-E in the CNS, suggesting that spongiform degeneration could be a direct result of viral gene expression in the neuron, rathe than an indirect consequence of viral infection of glia o endothelial cells[7,10]. It was unexpected that a post-mitotic cell would be the main target for Cas-Br-E and in this respect Cas-Br-E resembles the lentiviruses, which replicate in non-dividing macrophages[13,14]. The interaction of Cas-Br-E with neurons, however, differs in important ways from its interaction with other cells. Replication of Cas-Br-E in neurons, but not in non-neuronal cells, is abortive, by contrast with replication of the non-neurotropic MoMuLV which replicates productively in all cells. Abortive infection may explain the failure in previous

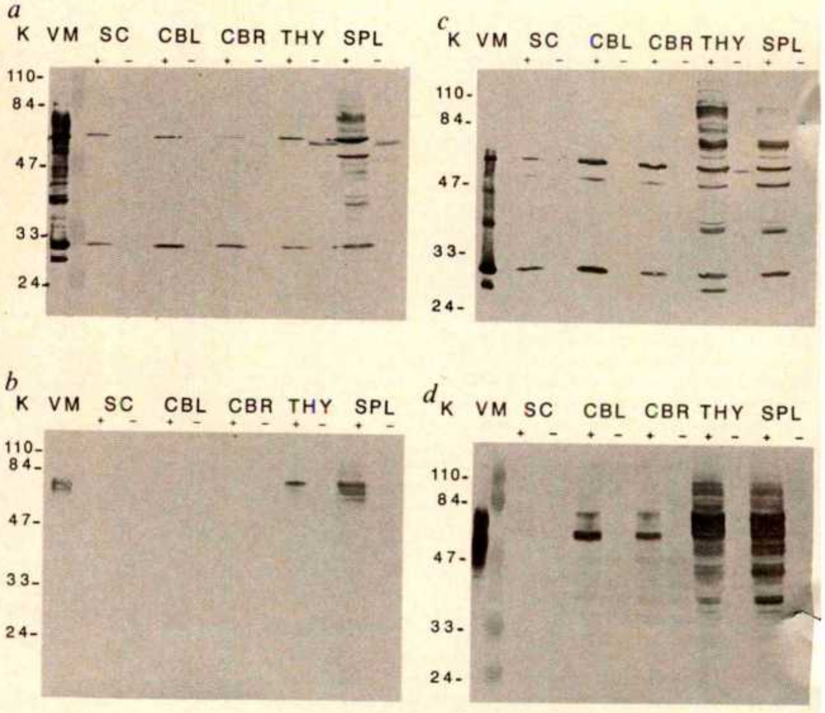

FIG. 2 Western blot analysis of tissue extracts prepared from Cas-Br-E (*a* and *b*) and MoMuLV-infected (*c* and *d*) mice and probed with anti-total virus (*a* and *c*) or anti-Env (*b* and *d*) antisera. Note that Env protein was not detected in Cas-Br-E-infected CNS but was present in other Cas-Br-E infected tissues and in all MoMuLV-infected tissues. Prominent bands with anti-total virus serum correspond to Pr65gag and p30. +, infected tissue; −, uninfected control tissue; sc, spinal cord; cbl, cerebellum; cbr, cerebrum; thy, thymus; spl, spleen; v, purified virus; m, protein relative molecular mass marker.

METHODS. Tissues were teased apart in 4 °C PBS. Cytoplasmic extracts were prepared by resuspending tissue in lysis buffer (1:5, vol cells:vol buffer; 300 mM sucrose, 1 mM EDTA, 1 mM EGTA, 20 mM Tris buffer, pH 8.0, 20 mM HEPES buffer, pH 7.3, 0.4% Nonidet-P40, 50 mM NaCl, 2 mM PMSF, 1 unit ml^{-1} aprotinin, 5 $\mu\mathrm{g\ ml}^{-1}$ chymostatin, 5 $\mu\mathrm{g\ ml}^{-1}$ pepstatin and 5 $\mu\mathrm{g\ ml}^{-1}$ leupeptin) and centrifuging at 400*g* for 5 min. Supernatants were diluted 1:1 in 2× Laemmli sample buffer and frozen at −70 °C. Aliquots (100 μl) of each extract were run on 12.5% polyacrylamide gels and transferred to nitrocellulose[19]. Nitrocellulose membranes were blocked with 5% Carnation milk, probed with goat anti-total virus (NIH81S107) or goat anti-Env (W. Schafer antibody or NIH78S225) antisera diluted in PBS containing 0.3 M NaCl and 0.1% Triton X-100 (Buffer A) for 4 h, washed in Buffer A, then incubated with rabbit anti-goat AP conjugate (Sigma, 1:2,000) for 30 min. Filters were washed with buffer A and developed in 1 mg ml^{-1} nitroblue tetrazolium, 0.5 mg ml^{-1} BCIP in 50 mM diethylamine, pH 9.5 containing 10 mM $\mathrm{MgCl_2}$.

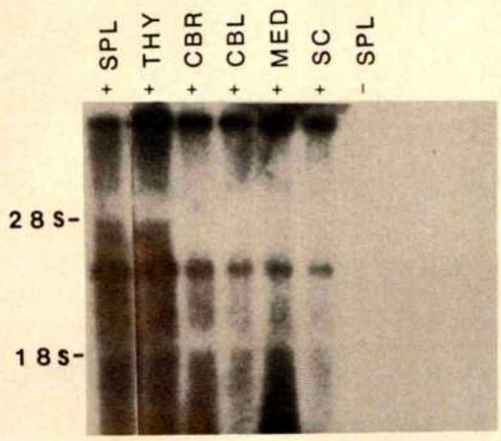

FIG. 3 Northern blot analysis of RNA from Cas-Br-E mice. +, infected tissue; −, uninfected control tissue. Abbreviations as in Fig. 2; med, medulla. The +Spl lane was exposed half as long as other lanes.

METHODS. Total RNA was isolated from tissue obtained from a 3-month-old mouse infected with Cas-Br-E neonatally by acid guanidinium-thiocyanate-phenol-chloroform extraction[20] and separated on 1% agarose, 2.2 M formaldehyde gels. RNA was transferred by capillary blotting onto Gene Screen Plus nylon membranes (NEN). Filters were probed with a Cas-Br-E-sequence-specific probe (*Bam-Taq* fragment of *env*). DNA inserts of plasmid were purified, radiolabelled with ^{32}P by random primer synthesis[21] and hybridized to northern blots at a concentration of 2.5×10^6 c.p.m. ml^{-1}. Hybridizations were carried out at 47 °C in 50% formamide, 5× SSC, 2× Denhardt's and 0.01% yeast transfer RNA. After overnight hybridization, filters were washed twice at 70 °C in 2× SSC, 0.1% SDS, then washed twice in 0.2× SSC, 0.1% SDS at 70 °C, and exposed.

studies to detect mature particles in neurons of infected animals.

Although Cas-Br-E viral Gag and Pol proteins were efficiently translated from viral genomic RNA in all tissues, no Env proteins were detected in brain and spinal cord, although normal amounts of *env* mRNA were synthesized. Although these results indicate that the Cas-Br-E *env* mRNA is present, they do not rule out the possibility that a splicing error not grossly affecting mRNA size has occurred. Alternatively, neuron-specific post-translational steps in Env synthesis could be impaired, leading to aberrant Env synthesis in the CNS, resulting in accumulation of viral products detrimental to normal cell function or interfering with the processing of normal cellular glycoproteins, altering cell metabolism and/or viability.

Our results are intriguing in the light of human retroviral neurological diseases. Abortive replication of virus in neurons and/or glial cells could be involved in their pathogenesis. Similarities between human immunodeficiency virus (HIV) and human T-cell leukaemia virus (HTLV-1) neurological diseases and non-inflammatory neurodegenerative diseases of unknown aetiology (such as Alzheimer's disease and Creutzfeld-Jacob disease) have been pointed out and a retroviral aetiology suggested[15,16]. It may be that these diseases involve retroviruses, but because viral replication is abortive, viral infection of neurons and/or glial cells may be difficult to detect. Thus, our results give impetus to the investigation of abortive retroviral infection in the aetiology of these diseases. □

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T-cell recognition of an immunodominant myelin basic protein epitope in multiple sclerosis

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MULTIPLE sclerosis is thought to be an autoimmune disease of the central nervous system mediated by T cells specific for a myelin antigen[1,2]. Myelin basic protein has been studied as a potential autoantigen in the disease because of its role as an encephalitogen in experimental autoimmune encephalomyelitis and post-viral encephalomyelitis[3,4] and because of the presence in the blood of multiple sclerosis patients of *in vivo*-activated T cells reactive to myelin basic protein[5]. Immune involvement in multiple sclerosis has been further suggested by the association with the major histocompatibility complex class II phenotype DR2, DQw1 (refs 6–9). To define the T-cell specificity toward myelin basic protein, 15,824 short-term T-cell lines were established from multiple sclerosis subjects, subjects with other neurological diseases, and normal controls. Here we report a higher frequency of T-cell lines reactive with a DR2-associated region of myelin basic protein between residues 84–102 in patients with multiple sclerosis compared with controls. A second region, identified between residues 143–168, was recognized equally in multiple sclerosis patients and controls and was associated with the DRw11 phenotype. These DR2 and DRw11 associations were also observed among T-cell lines generated from family members of a multiple sclerosis patient. The immunodominant 84–102 peptide from myelin basic protein was both DR2- and DQwl-restricted among different T-cell lines. These results raise the possibility that this immunodominant region may be encephalitogenic in some DR2⁺ individuals.

A barrier to studying the fine specificity of reactivity to myelin basic protein (MBP) in multiple sclerosis (MS) has been the low level of reactivity in standard proliferation assays[10,11]. To obtain the necessary number of cells for analysis, short-term T-cell lines (15,824 in total) were generated from 51 subjects by culturing peripheral blood mononuclear cells with MBP and then adding interleukins-2 and -4 (IL-2 and IL-4). IL-4 increases the growth rate of T-cell clones[12] and 2.5×10^5–5.0×10^5 T-line cells were generated from every well plated to perform the required analysis. On day 13, each line was tested for reactivity to MBP; reactive lines were then tested for reactivity to overlapping 20-residue oligopeptides encompassing the human MBP sequence. For MHC restriction experiments, lines reactive to

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an MBP peptide were restimulated for two more cycles, first with MBP and then with the specific MBP fragment recognized by that line. In a subgroup of patients, the frequency of T cells recognizing proteolipid apoprotein (PLP), another major encephalitogenic central nervous system antigen, was investigated[13,14].

Frequency analysis for MBP and PLP reactivity was performed on patients with definite, early relapsing-remitting MS, subjects with other neurological diseases with central nervous system tissue damage and normal subjects, age and sex matched to the MS patients (Table 1). Although the frequency of MBP-reactive lines was slightly higher in subjects with MS compared with the other subjects, this was not statistically different. There was more reactivity to PLP in patients with MS compared with those with other neurological diseases, but this did not reach statistical significance.

Of a total of 302 lines from patients with MS that could be expanded and confirmed to react with MBP on repeated analysis, 140 (46.4%) reacted with MBP residues 84-102. In the control groups, 11 of a total of 100 MBP-reactive T-cell lines (11.0%) recognized this MBP peptide. We calculated the actual frequency of T cells derived from the peripheral blood that reacted with each MBP peptide for each individual subject. The mean values of patients with MS and the control subjects are shown in Fig. 1*a*. The frequencies of peptide-specific lines from normal subjects and from other neurological disease controls were virtually identical and thus were combined for analysis. The mean frequency of T-cell lines selectively reactive to MBP residues 84-102 was higher in subjects with MS compared with controls, although there were no differences among groups in the relative stimulation indices of T-cell lines reacting to MBP peptides. Significant but less striking increases in the frequency of reactivity to MBP residues 61-82 and 124-142 were also observed in MS patients, whereas both MS and control subjects showed high frequencies of T-cell lines reactive with MBP residues 143-168.

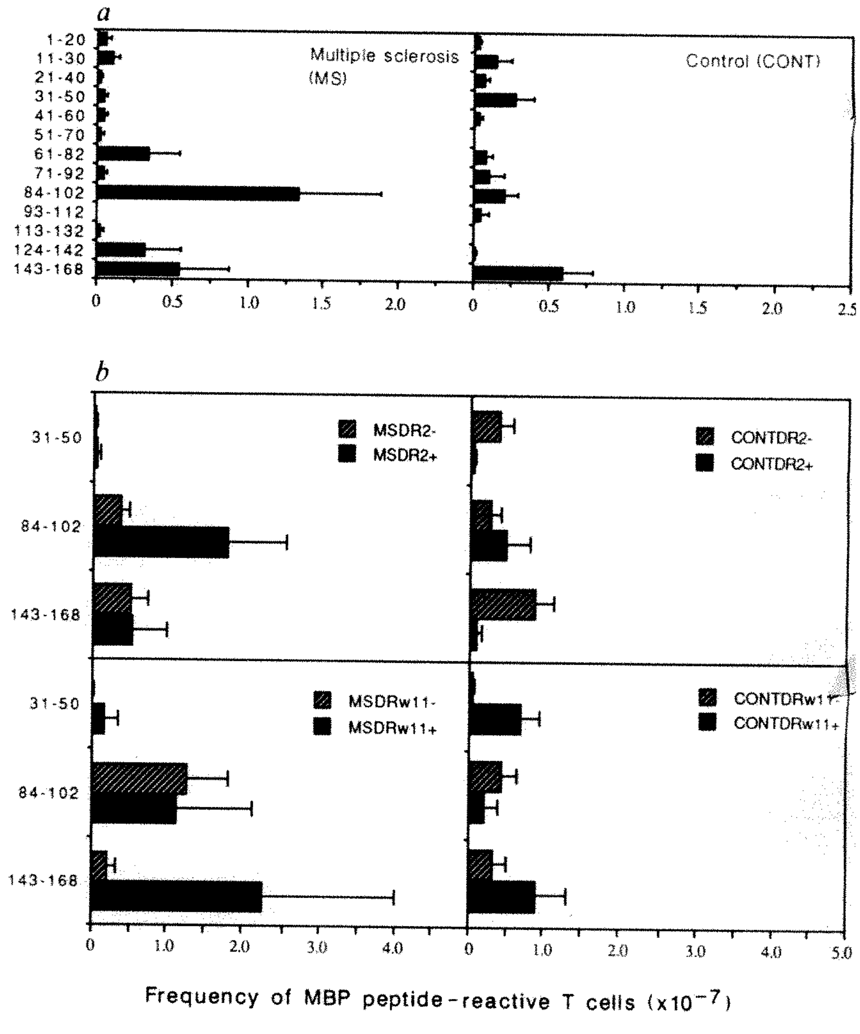

FIG. 1 *a*, Mean frequency of peripheral blood T cells recognizing overlapping synthetic peptides of human MBP in 23 MS patients and 16 randomly selected controls (10 subjects with other neurological diseases of the central nervous system and 6 normal controls). For each patient, 288 wells were plated with 2×10^5 blood mononuclear cells (MNC), separated by Ficoll density gradient sedimentation, in 96-well round-bottom plates. MNC were cultured with MBP (10 μg ml^{-1}) in media consisting of RPMI 1640, 10% pooled human serum, 1% HEPES buffer, 1% penicillin/streptomycin and 2% glutamine for 13 days. On day 3 and then every 3-4 days, 5% IL-2 (ABI, Columbia, MD) and 2 U ml^{-1} of rIL-4 (Genezyme, Boston) were added to the culture. On day 13, an aliquot of each T-cell line was analysed for reactivity to MBP by placing 10,000 cells from each T-cell line with 10,000 irradiated autologous MNC as antigen-presenting cells (APC) in duplicate for 72 h in a round-bottom 96-well microtitre plate and pulsing with [^{3}H]-thymidine during the last 18 h of culture. APC were prepared by pulsing 10^7 APC in 1.0 ml media with or without 100 μg MBP for 1 h at 37 °C. Lines positive for proliferation to MBP (defined as showing both a stimulation index of >3 and a Δc.p.m. of >500) were re-tested 72 h later for reactivity to 13 different peptides (concentration of antigen 100 μg peptide ml^{-1}) encompassing the human MBP sequence. MBP was extracted from human brain tissue, purified on a CM-52 column, and the highest relative molecular mass (M_r, 18,500) was used[24]. MBP peptides were synthesized by Biosearch Lab Inc., (San Rafael, California) using the solid phase method and were purified by HPLC. MBP peptide sequences are as follows (single-letter amino-acid code): 1-20, ASQKRPSQRHGSKYLATAST; 11-30, GSKYLATASTMDHARHGFLP; 21-40, MDHARHGFLPRHRDTGILDS; 31-50, RHRDTGILDSIGRFFGGDRG; 41-60, IGRFFGGDRGAPKRGSGKDS; 51-70, APKRGSGKDSHHPARTAHYG; 61-82, HHPARTAHYGSLPQKSHGRT; 71-92, SLPQKSHGRTQDENPVVHFF; 84-102 DENPVVHFFKNIVTPRTPP; 93-112, KNIVTPRTPPPSQGKGRGLS; 113-132, LSRFSWGAEGQRPGFGYGGR; 124-142, RPGFGYGGRASDYKSAHKG; 143-168, FKGVDAQGTLSKIFKLGGRD. In pilot experiments, plating 2×10^5 mononuclear cells per well gave frequencies of between 1 and 20% MBP-reactive lines. Thus according to the Poisson probability distribution, most MBP-reactive T-cell lines represent a single MBP-reactive T cell. As 2×10^5 MNC were plated for each of 288 wells per subject, the actual frequency of MBP-reactive T cells can be calculated by dividing the frequency of MBP reactive lines (Table 1) by 2×10^5. The frequency of T cells reactive with specific MBP synthetic peptides was calculated by multiplying the frequency of MBP-reactive T cells for each subject by the proportion of lines reactive with each peptide on the second analysis for that same subject. Specifically, the frequency of circulating T cells reactive with synthetic peptide is equal to the frequency of MBP-reactive T cells × (no. T-cell lines reactive with synthetic peptide)/(Total no. MBP reactive lines on 2nd analysis). The mean frequency ±s.e.m. for each peptide for patients with MS, normal controls and subjects with other neurological diseases were calculated. Values for both control groups were combined. *b*, Frequency of MBP peptide-reactive lines in relation to class II MHC expression in patients with MS and in controls. An additional 6 DR2$^+$ normal controls were studied in addition to the original 16 control subjects and 23 patients with MS. Subjects with the DR2 phenotype had a higher proportion ($P=0.003$) and frequency ($P=0.045$) of T cells recognizing MBP residues 84-102. Subjects with the DRw11 phenotype had a higher frequency ($P=0.006$) of T cells recognizing MBP residues 143-168 (unpaired *t*-test).

TABLE 1 Subject characteristics and frequency analysis of MBP- and PLP-reactive lines

	Age	Sex (%) (m/f)	MHC (%) DR2	DR4	DRw11	DQw1	No. antigen reactive lines/total no. lines MBP	PLP	Mean frequency of antigen-reactive lines (%) MBP	PLP
Multiple Sclerosis ($n=23$)	34.2±1.4	35/65	60.9	26.1	13.0	78.2	554/7746	20/432	7.18±2.38	3.34±1.56
Other Neurological disease ($n=10$)	38.7±3.2	43/57	14.3	0.0	42.9	85.7	118/2880	3/384	4.10±1.04	0.90±0.62
Normal ($n=6$)	30.3±1.5	50/50	16.7	0.0	50.0	66.6	73/1742	ND	4.70±1.58	ND
DR2$^+$ Controls ($n=6$)	32.0±2.9	50/50	100	16.7	0.0	100	53/1728	ND	3.08±2.06	ND

Patients with MS were Caucasian and had well-characterized early relapsing remitting disease with minimal disability with at least two exacerbations within the previous 24 months and positive lesions on MRI at the time of blood drawing. Subjects with other central nervous system diseases had the following diagnoses: 1-3 weeks after either cerebrovascular accident (4); brain trauma with central nervous system haemorrhage (4); metastatic brain tumour (2). The total number of lines reactive with either MBP or PLP and the total number of T-cell lines generated are shown. The frequencies of MBP- and PLP-reactive lines were calculated separately for each subject by dividing the number of MBP-reactive lines by the total number of lines generated and the mean value ±s.e.m. are given. See Fig. 1 legend for methods for T-cell cloning. ND, not determined.

TABLE 2 MHC restriction of T-cell lines reactive to MBP 84–102

[^{3}H]thymidine incorporation (Δ c.p.m.) of T-cell lines

(a)

MHC phenotype of APC DR	DQw	Hy. 1A8 APC	MBP	Peptide 84–102	Hy. 2C9 APC	MBP	Peptide 84–102	Hy. 2E11 APC	MBP	Peptide 84–102	Hy. 2H9 APC	MBP	Peptide 84–102	Hy. 3A10 APC	MBP	Peptide 84–102
2, 7	**1, 3**	32	21, 192	10, 747	83	3, 263	14, 991	148	18, 593	30, 368	169	2, 797	10, 444	139	6, 887	24, 411
2	**1**	83	56	32	82	78	112	217	52, 939	49, 399	636	327	658	23	28	26
4, **7**	2, **3**	32	26	53	45	55	142	37	167	81	226	258	263	306	719	915
3	2	46	32	52	43	44	110	101	98	349	769	402	1,973	23	31	100
3, 10	**1**, 2	35	30, 737	49, 144	158	25	80	36	58	42	49	54	46	42	22, 823	31, 121
2, **7**	**1**, 2	38	40	47	43	39	43	78	53, 441	32, 357	261	190	289	33	19	36
7, w11	2, 7	44	39	54	57	124	259	34	25	33	51	58	97	967	1, 214	2,744

(b)

Monoclonal antibodies		Hy. 1A8	Hy. 2C9	Hy. 2E11	Hy. 2H9	Hy. 3A10
	APC alone	32	83	39	50	139
Specificity	None	10,747	14,991	3,325	8,659	24,411
	Control	11,375	15,322	4,131	8,156	27,363
Anti-DR	PL8	11,051	41	31	142	25,016
	L243	16,792	586	22	36	21,148
	6SP4.1	19,119	405	46	92	26,412
Anti-DQ	1A3	4,851	11,444	2,102	5,446	15,714
	Tu22	1,189	13,442	1,073	7,661	13,488
	Leu10	1,128	14,924	2,255	7,678	13,090
Anti-DP	B7/21	7,917	15,922	2,337	6,689	23,452
Anti-DR + DP	Tu35	13,606	75	21	42	27,104

(*a*) Proliferation of T-cell lines using a panel of different MNC as APC are shown. Five T-cell lines reactive to MBP residues 84–102 from subject Hy were expanded by repeated cycles of stimulation with autologous irradiated MNC pulsed with synthetic MBP peptide 84–102 and examined for recognition of this region of MBP and human MBP. Briefly, 50,000 T-line cells were plated in triplicate with 50,000 irradiated APC MNC for 72 h in round-bottom 96-well microtitre plates and wells were pulsed with [^{3}H]thymidine for the last 18 h culture. APC MNC were either cultured alone, pulsed with 100 μg ml^{-1} synthetic peptide 84–102 (the optimal concentration of peptide to induce proliferation), or pulsed with 100 μg ml^{-1} MBP. The average c.p.m. values for triplicate wells are shown. DR and DQw haplotypes are given and haplotypes common with the patient (top line), who was DR2, DR7, DQw1, DQw3, are in bold. (*b*) Inhibition of proliferation by class-specific anti-MHC monoclonal antibodies. The panel of five T-cell lines reactive with MBP residues 84–102 were plated with autologous APC MNC either alone or in the presence of APC pulsed with synthetic peptide 84–102, as above, in the presence of monoclonal antibodies (final concentration 1:100) recognizing different MHC class II gene products. Control was an irrelevant monoclonal antibody. Antibody nomenclature according to the 10th International Histocompatibility Workshop[25].

As previously reported[6-9], the DR2, DQw1 phenotype was infrequent in the controls and more common in MS patients (Table 1). An association was observed between the DR2 phenotype and both the proportion and the frequency of T-cell lines reactive to MBP residues 84–102 (Fig. 1*b*). To determine whether there was also this association in non-MS subjects, an additional six normal subjects with the DR2, DQw1 phenotype were investigated. An association with DR2 was also found among controls in terms of the proportion of T-cell lines reactive with MBP residues 84–102 (DR2$^+$ controls, 31.0±10.8%; DR2$^-$ controls, 10.1±0.4%). The total frequency of lines reactive with this region of MBP was, however, less than that in patients with MS (Fig. 1*b*). Although DQw1 is in linkage disequilibrium with DR2, independent analysis of peptide reactivity revealed no association with DQw1 phenotype. The lack of a DQw1 association in subjects with MBP peptide 84–102 reactivity may, however, also relate to differences in DQw1 polymorphisms that were not examined in the present study.

The DRw11 phenotype was more common in controls than in subjects with MS (Table 1). Its expression was associated with a higher frequency of lines reactive to MBP residues 143–168 in both MS patients and controls (Fig. 1*b*). Reactivity to MBP residues 31–50, which was predominantly observed in controls, was also associated with DRw11. Other MHC associations were not found.

It was important to determine the MHC restriction of the

TABLE 3 Relationship between MHC expression and frequency of T cells reactive to immunodominant MBP epitopes in family with DR2 and/or DRw11 phenotype

	Patient	P1	P2	S1	S2	S3
	DR2 **DQw1**	DR4 DRw53 DQw3	**DR2** **DQw1**	DR4 DRw53 DQw3	DR4 DRw53 DQw3	**DR2** **DQw1**
MBP peptide	**DRw11** **DRw52** **DQw1**	**DRw11** **DRw52** **DQW1**	DRw6 DRw52 DQw1	DRw6 DRw52 DQw1	DRw6 DRw52 DQw1	DR4 DRw53 DQw3
84-102	**49**	1	**4**	**6**	1	**7**
143-168	**41**	**14**	0	3	2	2

The family members of an MS patient expressing the DR2, DQw1; DRw11, DRw52, DQw1 class II MHC haplotypes were examined for the frequency c T-cell lines reactive with MBP residues 84-102 and 143-168. MNC (2×10^5) in each of 288 wells (three 96-well round-bottom plates) were cultured wit MBP (10 μg ml^{-1}) as outlined above for each subject. On day 16, each T-cell line was analysed for reactivity to synthetic peptides corresponding to MB residues 84-102 and 143-168. The number of lines reactive with each peptide (SI > 3, Δc.p.m. > 500) generated per subject are shown. The actu stimulation indices were generally >20. P1 and P2, parents; S1-S3, siblings.

T-cell lines reactive with the immunodominant MBP epitope. Monoclonal antibody blocking studies of five T-cell lines reactive with MBP residues 84-102 suggested that both DR and DQ molecules could function as restricting elements. Among clones blocked by anti-DR monoclonal antibody, clone 2E11 proliferated in response to MBP residues 84-102 with the panel of DR2$^+$ antigen-presenting cells (APC) whereas 2C9 and 2H9 proliferated only with autologous APC (Table 2). The recognition of peptide by clones 1A8 and 3A10, which were partially blocked by anti-DQ monoclonal antibodies, was restricted to APC from the responder and one of two APC donor subjects expressing DQw1.

To investigate the relationship between MHC expression and frequency of T-cell reactivity to immunodominant MBP epitopes, a family with one afflicted sibling of both DR2 and DRw11 phenotypes was studied (Table 3). A total of 1,728 individual T-cell lines were generated from both parents and four siblings and the number of lines reactive with either MBP peptide 84-102 or 143-168 was determined. The DR2$^+$, DRw11$^+$ patient had a high frequency of T-cell lines reactive to both MBP residues 84-102 and 143-168. The DRw11$^+$ parent preferentially recognized MBP residues 143-168, whereas the DR2$^+$ parent preferentially recognized MBP residues 84-102. The frequency of MBP peptide reactive lines, however, was lower than that of the patient. One sibling was DR2$^+$ and preferentially recognized MBP residues 84-102. Of two HLA-identical siblings (DR4, DQw3/DRw6, DQw1), one preferentially recognized MBP peptide 84-102. Although DR4 or DQw1 may also restrict recognition of MBP residues 84-102, other factors such as inherited T-cell receptor polymorphisms may have influenced T-cell reactivity to the MBP autoantigen in one of the DR4, DQw3/DRw6, DQw1 siblings. In this regard, DR4 was also more frequent in our series of MS patients compared with control subjects. This family linkage analysis suggests that optimum recognition of immunodominant MBP eptitopes requires specific class II MHC alleles both in patients with MS and controls. In total, these studies indicate that although control subjects expressing DR2 seem preferentially to recognize the same MBP determinant as do DR2$^+$ MS patients, their clonal frequency in the blood is less than that of patients with MS.

Previous studies using peripheral blood have suggested there is a response to a number of MBP regions, in particular 83-96, which contains an epitope that overlaps with the immunodominant 84-102 MBP region identified here[15]. In those studies, however, the standard 7-day-proliferation assay did not detect peptide reactivity in controls. The presence of T cells reactive with MBP residues 84-102 in DR2$^+$ normal subjects suggests that other immune mechanisms exist to prevent the activation of circulating autoreactive T cells. Although in the present study MBP-reactive T cells could be generated by culture *in vitro* from controls to the same degree as patients with MS, recent work indicates that MBP-reactive T cells are activated only in patients with MS and not in controls[5]. Other factors involved in the development of autoimmune diseases may relat to the altered systemic immunoregulation seen in MS a evidenced by decreased levels of antigen-nonspecifi suppression[16,17].

In experimental autoimmune encephalomyelitis (EAE), a single immunodominant region of MBP influenced by class I MHC has been observed among different rat and mous strains[18-21] and these peptides are the major encephalitogen i the animal strain is susceptible to EAE[22]. In determing the relationship between MHC and encephalitogenic immunodominant regions of MBP, the animal need not have had EAE. Unprimed Lewis rat lymph-node cells selectively react with immunodominant MBP residues 48-88 and these T-cell clones are encephalitogenic[23]. The present experiments in humans define immunodominant regions of human MBP that are linked to a particular MHC. As the DR2, DQw1 phenotype is most common in patients with MS[6-9], the T-cell reactivity to MBP residues 82-102 in association with this phenotype may be analogous to the encephalitogenic T cells defined in EAE.

To show that MS is a cell-mediated autoimmune disease analogous to EAE, certain criteria can be proposed. First an association should exist between an immunodominant region of the presumed autoantigen and disease-associated class II MHC haplotypes. Second, there should be an increase in frequency of T cells that react with this immunodominant epitope. Finally, the course of the disease must be altered by elimination of autoreactive T cells or by inducing immune tolerance to the autoantigen identified in the first two criteria. This final condition implies that *in vitro* experiments on their own cannot prove the association of an autoantigen with a disease and instead clinical trials are necessary.

Our results have begun to address the first two criteria, showing an association between immunodominant regions of human MBP with the DR2 class II MHC phenotype and an increased frequency of T cells recognizing the MHC-linked immunodominant epitope of the MBP autoantigen in MS. In addition, PLP might also serve as an autoantigen. If MBP is a target autoantigen in MS, our results imply that an immunodominant region of MBP, between residues 84-102, is an encephalitogenic region in some DR2$^+$ individuals. In non-DR2 individuals other immunodominant MBP epitopes or other antigens may be associated with the disease.

Note added in proof: Since submitting our article, two works have come to our attention demonstrating similar immunodominant regions of human MBP: Martin, R. *et al.*, *J. Immunol* (in the press); Pette, M., *Proc. natn. Acad. Sci. U.S.A.* (in the press). □

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Activated CD8 binding to class I protein mediated by the T-cell receptor results in signalling

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THE CD8 glycoprotein of T cells binds nonpolymorphic regions of class I major histocompatibility complex proteins on target cells[1,2] and these interactions promote antigen recognition and signalling by the T-cell receptor[3–11]. Studies using artificial membranes indicated that effective CD8/class I interaction is critical for response by alloantigen-specific cytotoxic T lymphocytes when class I protein is the only ligand on the antigen-bearing surface[10,11]. But significant CD8-mediated binding of cytotoxic T lymphocytes to non-antigenic class I protein could not be detected in the absence of the alloantigen[12]. These apparently contradictory findings indicate that CD8 binding to class I protein might be activated through the T-cell receptor and the results reported here demonstrate that this is the case. Treatment of cytotoxic T lymphocytes with soluble anti-T-cell receptor antibody activates adhesion of the cytotoxic T lymphocytes to class I, but not class II proteins. The specificity of this binding implies that it is mediated by CD8 and blocking by anti-CD8 antibodies confirmed this. Furthermore, binding of CD8 to class I protein resulted in generation of an additional signal(s) necessary to initiate response at low T-cell receptor occupancy levels.

Purified class I alloantigen can be immobilized on plastic and stimulates T-cell receptor (TCR) triggered degranulation by cloned cytotoxic T lymphocytes (CTL)[11]. Specific binding of ^{51}Cr-labelled CTL to the immobilized alloantigen can be measured (Fig. 1). Roughly 15–20% of C11 CTL, specific for H-2K^b, were bound following a 2-h incubation at 37 °C with immobilized K^b antigen. Nonspecific binding in wells blocked with fetal calf serum (FCS) or having immobilized class II (I-A^k) protein was only ~2%. No binding above this background level was found when non-antigenic class I proteins (H-2D^b or D^d) were immobilized. In numerous experiments, specific binding of 15–30% of the CTL to alloantigen has been observed, consistent with studies examining target-cell binding by cloned CTL (refs 13, 14).

To determine if CTL binding to irrelevant class I protein could be activated by the TCR, binding was measured after treating CTL with anti-TCR monoclonal antibody F23.1 in solution. Soluble F23.1 stimulated CTL binding to immobilized class I, but not to class II major histocompatibility complex (MHC) proteins (Fig. 1). Binding occurred equally well to the class I protein borne by the CTL (D^d) or to third-party class I protein (D^b) and was comparable to that obtained to alloantigen (K^b) in the absence of soluble anti-TCR monoclonal antibody. Stimulation of binding was specific for anti-TCR monoclonal antibody. Addition of irrelevant antibodies or antibodies specific for several other CTL surface proteins including H-2K^k, CD8 or Thy-1 did not trigger binding to class I proteins (data not shown). Binding to irrelevant class I proteins was detectable within minutes of adding anti-TCR antibody and centrifuging the cells into the wells, increased to 15–30% over the next hour, and then remained stable. Similar kinetics were observed for binding to alloantigen in the absence of soluble anti-TCR antibody (data not shown).

Neither soluble anti-TCR monoclonal antibody nor immobilized class I protein alone (other than the alloantigen) trigger CTL degranulation. Degranulation is triggered, however, by the combination of soluble anti-TCR antibody and immobilized class I proteins. As for binding, triggering of degranulation is

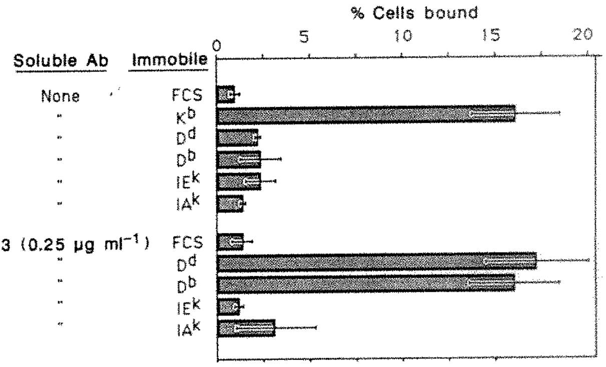

FIG. 1 Soluble anti-TCR monoclonal antibody triggers CTL binding to irrelevant class I protein. The indicated MHC proteins were immobilized (0.25 μg per well) and the wells blocked using 2% FCS. Binding by C11 was determined as described below using a 2.5-h incubation at 37 °C. Where used, soluble anti-TCR monoclonal antibody (F23.1) was added to the CTL suspension (final concentration 0.25 μg ml^{-1}) immediately before initiating the binding assay. For this experiment, total ^{51}Cr incorporation was 26,428 c.p.m. per 10^5 cells, and spontaneous release was 815 c.p.m. per 10^5 cells.

METHODS. Cloned CTL: The cloned CTL lines used included C6, C11 and C13. Characterization and maintenance of these have been described[11]. All were derived from B10.BR × B10.D2F1 (H-2^k × H-2^d) responder mice and were specific for the H-2K^b alloantigen. MHC protein purification and immobilization: MHC class I and II proteins were purified by monoclonal antibody affinity chromatography and immobilized on plastic as previously described[11,31]. In brief, purified antigen in 0.5% deoxycholate was diluted in PBS, pH 7.2, to a final concentration of 1 or 2 μg ml^{-1}, added to wells of a plastic microtitre plate (Falcon 3912 Flexible Assay Plate), allowed to bind 1.5 h at 25 °C and unbound protein then removed. Then 2% FCS was added to the wells for 0.5 h to block unoccupied sites. Assay of CTL binding: CTL were labelled by incubating for 1 h at 37 °C using 100 μCi Na^{51}Cr per 10^7 cells. Cells were then washed and resuspended in RPMI medium containing 2.5% newborn calf serum (10^6 per ml). Binding was initiated by adding 10^5 cells per well in 0.1 ml and centrifuging at 1,200 r.p.m. for 1 min. Plates were then incubated for 1–2.5 h at 37 °C in 5% CO$_2$. After this, 0.1 ml PBS per well was added, plates placed on an ice water bath for 10 min and unbound cells removed by pipetting 10 times with a Pipetman (Gilson, Inc.). Well bottoms were then cut off and radioactivity levels determined. Spontaneous release of ^{51}Cr was determined for cells incubated under identical conditions. Binding was calculated as percentage cells bound = 100 × (c.p.m. bound)/(total c.p.m. − spontaneous c.p.m.). Visual counting confirmed that this procedure provided an accurate measurement of bound cells. Antibodies used: the monoclonal antibodies used in this study were F23.1 anti-TCR, 145-2C11 anti-T3, 53-6.7 anti-Lyt-2 (CD8), YTS 169.4 anti-Lyt2, 31-68.8 anti-Lyt-2, HO-13-4 anti-Thy-1.2, 11-4.1 anti-H-2K^k and anti-SASD, a murine IgG specific for a chemical hapten. The sources and purification of these antibodies have been described previously[11].

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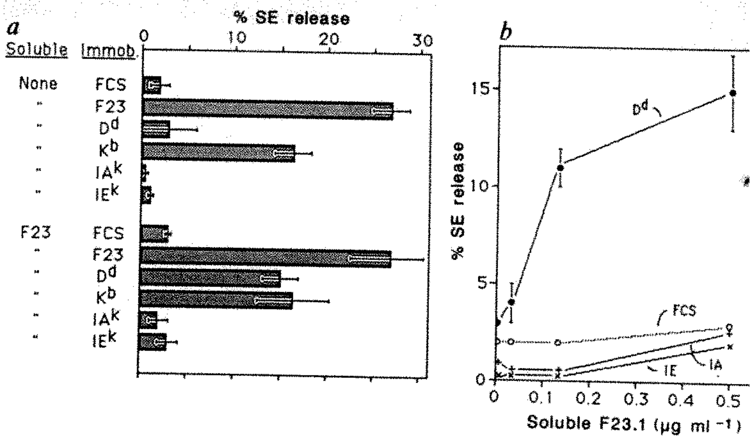
FIG. 2 CD8 binding to class I protein provides signalling necessary for the degranulation response. *a*, C11 CTL (10^5 per well) were added to wells containing the indicated immobilized protein (Immob.). When used, F23.1 anti-TCR monoclonal antibody was added to the CTL suspension at a final concentration of 0.5 μg ml^{-1} immediately before CTL were added to the wells. Cells were centrifuged and plates incubated for 2.5 h at 37 °C. After this, aliquots of supernatant were removed and assayed for serine esterase (SE) activity as previously described[11]. *b*, Triggering of serine esterase release was determined as described above, except the concentration of soluble F23.1 monoclonal antibody was varied as indicated. Wells contained immobilized H-2D^d (●), I-A^k (+), I-E^k (×), or FCS (○).



specific for class I (H-2D^d) and not class II (I-A^k) proteins (Fig. 2) and is dependent on the level of anti-TCR monoclonal antibody present (Fig. 2*b*). Additional experiments have shown that D^d, D^b and K^k class I proteins are all effective and that response occurs only when the soluble antibody is directed against the TCR. Thus, binding of CTL to class I protein, triggered by the TCR, results in generation of an additional signal(s) for the response.

The binding and class I-dependent response detected in these experiments is probably mediated by CD8; the alternative is that CTL have an additional, unidentified receptor that specifically binds class I conserved determinants. Although TCR may have a low affinity for self-MHC, there is no apparent contribution from this, as class I proteins are bound and stimulate equally well whether or not they are the same as those present on the responder cells. Further evidence that CD8 mediated the binding was that soluble anti-CD8 antibody blocked binding to class I protein triggered by soluble anti-TCR antibody (Fig. 3), although control antibodies including anti-Thy-1 did not (data not shown). Anti-CD8 antibody also effectively blocked degranulation in response to the combination of soluble anti-TCR antibody and class I protein. By contrast, immobilized anti-TCR antibody stimulated a comparable level of degranulation but the response was only marginally affected by anti-CD8 antibody (Fig. 3), indicating that little or no anti-CD8-induced negative signalling was occurring under the conditions used.

These results indicate that perturbation of the TCR generates a signal to activate CD8 binding to class I protein. Several phosphorylation events occur on T-cell triggering, including phosphorylation of the CD8 protein[15]. Protein kinase inhibitors K252a and staurosporine inhibited anti-TCR-triggered binding to class I protein (Fig. 4) with 50% inhibitory concentrations (IC$_{50}$s) similar to those found in studies directly examining inhibition of kinase activities. In addition to inhibition of binding, degranulation was inhibited (data not shown). Thus a kinase, possibly protein kinase C, is likely to be involved in TCR-dependent activation of CD8 binding. A kinase may also be involved in the signalling resulting from CD8/class I binding. CD8 is associated with p56^{lck} protein-tyrosine kinase[16], which can phosphorylate the ζ-subunit of the TCR complex. If CD8 binding to class I protein activates p56^{lck} to phosphorylate ζ-chain, as seems to occur on CD4 cross linking[17], this might modify signalling occurring through the occupied TCRs and result in the initiation of response. A mechanism involving CD8 feedback to the TCR complex is compatible with the fact that a response can be triggered through the TCR without a CD8 ligand, as occurs with immobilized anti-TCR monoclonal antibody (Figs 2 and 3; refs 11, 18) or in CTL lacking CD8 (ref. 19).

Synergistic signalling through anti-TCR and anti-CD8 or anti-CD4 antibodies has been used to suggest that the most effective signalling involves a trimolecular complex, with TCR and CD8 (or CD4) interacting with the same MHC protein[20-24]. This mechanism could account for the fact that, in general, class I-restricted cells carry the CD8 antigen and class II-restricted cells carry the CD4 antigen. Formation of trimolecular complexes is unlikely in the experiments described here, as the TCR and CD8 do not use the same ligand. However, this does not eliminate the possibility that a physical association between TCR and CD8 is critical, and that this occurs even when the two receptors interact with different ligands.

Although CD8/class I binding by CTL was not observed in the absence of a TCR stimulus (Fig. 1), chinese hamster ovary (CHO) cells transfected with the CD8 α-chain gene bind to class I-bearing cells[1]. Binding by these TCR-negative cells was observed in the absence of any stimulus. But binding was observed only when very high surface levels of CD8 were expressed in these transfectants. Thus, it seems likely that CD8 has some affinity for class I protein in the unactivated state, but that this is detectable only at high surface densities of CD8. By contrast, with the CD8 surface density normally present on T

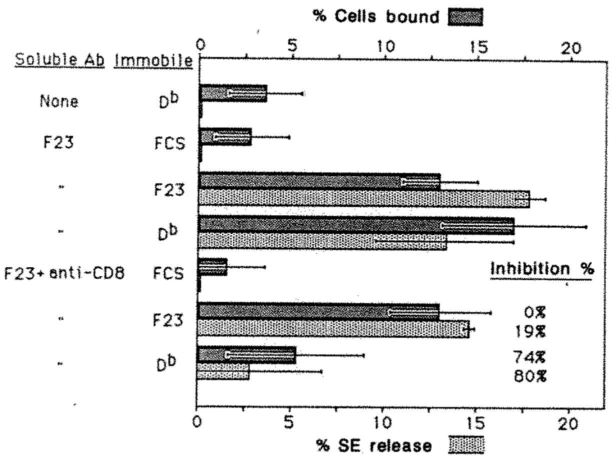


FIG. 3 Anti-CD8 antibody blocks TCR-activated binding and response to non-antigenic class I protein. The ^{51}Cr-labelled clone 11 CTL (10^5 per well) were incubated for 20 min at room temperature alone or with soluble F23.1 anti-TCR monoclonal antibody (.25 μg ml^{-1}). Where used, YTS 169.4 monoclonal antibody (as ascites) was then added at a 1:900 dilution. YTS 169.4 is a rat monoclonal antibody that recognizes a monomorphic murine Lyt-2 determinant[32]. Cells were then added to wells bearing the indicated immobilized protein, centrifuged and the plates incubated for 3 h at 37 °C. After this, aliquots of supernatant were removed and assayed for serine esterase (SE) activity[11]. Binding was then determined in the same wells as described (Fig. 1). Parallel comparisons of unlabelled and ^{51}Cr-labelled CTL have shown that ^{51}Cr-labelling has no effect on the degranulation response (data not shown). H-2D^b antigen used at 0.2 μg per well.

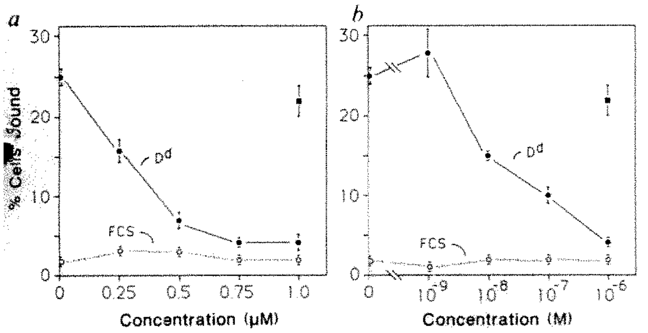


FIG. 4 Inhibition of TCR-activated CD8/class I binding by kinase inhibitors. Soluble F23.1 anti-TCR monoclonal antibody (0.25 μg ml^{-1}) was added to ^{51}Cr-labelled C11 CTL immediately before initiating binding to wells containing H-2D^d (0.1 μg per well; ●) or FCS (○). K252a (*a*) or staurosporine, (*b*) (Calbiochem) were added in dimethylsulphoxide (DMSO) (≤1% final concentration) to CTL 15 min before initiating binding. DMSO was added to solvent controls (■; 1% final concentration). Binding was for 2.5 h at 37 °C. Total ^{51}Cr incorporation was 40,298 c.p.m. Spontaneous release was determined at all drug concentrations and did not vary significantly. The average spontaneous release for all samples was 1,308 c.p.m.

cells, binding is only detected on activation through the TCR (Fig. 1).

Possible mechanisms by which TCR signalling might activate CTL binding mediated by CD8 include: rapid translocation of an internal pool of CD8 to the cell surface (preliminary results indicate that this does not occur on addition to CTL of soluble anti-TCR monoclonal antibody); induced microclustering of CD8, a mechanism implicated for PMA-activation of binding by C3bi receptors[25]; or conversion of CD8 from a low-affinity to a high-affinity state on TCR signalling. An example of the latter is provided by several hormone and growth-factor receptors whose affinity for ligand is modulated by phosphorylation[26]. These mechanisms are not mutually exclusive and more than one could contribute to activation of CD8 binding.

Results reported here demonstrate that CD8/class I interactions represent a TCR-activated adhesion-signalling system. Another adhesion system important in T-cell responses, that of LFA-1 and its I-CAM 1 and 2 ligands[27,28], is activated by crosslinking the TCR[29,30]. Whether the binding of LFA-1 to I-CAM mediates signalling events that contribute to TCR-dependent responses is not known. Adhesion-strengthening mechanisms triggered by TCR recognition of antigen, but distinct from TCR-antigen bonds, have been proposed, and Martz[28] has discussed in detail how such mechanisms can account for the properties of CTL/target cell interactions. It now seems that both LFA-1/I-CAM and CD8/class I represent such mechanisms, with at least the CD8/class I interaction also resulting in generation of a signal(s) for response. □

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Small G proteins are expressed ubiquitously in lymphoid cells and do not correspond to Bcl-2

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THE *bcl*-2 gene is consistently associated with t(14; 18) chromosomal translocations[1–6] observed in a large fraction of human B-cell lymphomas[7–8]. The t(14; 18) translocation results in deregulated expression of the *bcl*-2 gene[5] and synthesis of inappropriately high levels of the Bcl-2 protein[9,10]. Gene transfer studies suggest a role for Bcl-2 in cell survival[11,12], growth enhancement[13,14] and oncogenic transformation[11,15]. To test the suggestion that GTP-binding by Bcl-2 may mediate its biological effects[16] we characterized the GTP-binding proteins in lymphoid cells expressing Bcl-2. Expression of several small GTP-binding proteins was found to be ubiquitous and did not vary with levels of Bcl-2. By using immunological, electrophoretic and cell-fractionation techniques, we separated Bcl-2 from G proteins of small relative molecular mass (M_r) and showed that it is incapable of binding GTP. Our results show that small M_r G proteins are widely expressed in lymphoid cells and that Bcl-2 is not a novel member of this GTP-binding protein family.

The prevalence of small M_r G proteins in cells of the B-cell lymphoid lineage was investigated using a GTP-binding assay[17,18] that identifies the small M_r GTP-binding proteins but not the large G proteins with an $\alpha\beta\gamma$ subunit structure[19]. The cell lines examined corresponded to different stages of B-cell maturation and included pre-B cells (leukaemia line 697), mature B cells (lymphoma lines SU-DHL-4, DUL-5 and FL18) and plasma cells (murine plasmacytoma line J558L). In all lines, four small M_r GTP-binding proteins similar to those observed in NIH 3T3 cells were detected (Fig. 1). A fifth GTP-binding protein of M_r 21,000 (21K), recognized by the Ras11 monoclonal antibody[20], was also found in some lines.

In spite of the uniform expression of small M_r G proteins in these B-cell lymphoma cell lines, there were marked differences in Bcl-2 expression (Fig. 1), which ranged from very high levels in FL18 to undetectable levels in SU-DUL-5. There was also no obvious increase in GTP-binding activity or additional GTP-binding proteins in cell lines that overexpressed Bcl-2, or in the *bcl*-2-transfected EP5 cells compared with their non-transfected counterpart J558L (Fig. 1). These observations suggested no correlation between levels of GTP-binding and Bcl-2 expression.

The GTP-binding potential of Bcl-2 was more directly assessed after immunoprecipitation from total cellular extracts. Bcl-2 was the only protein specifically precipitated with anti-Bcl-2 antibodies as compared with pre-immune serum (Fig. 2*a*). The immune pellets and supernatant fractions were analysed for the presence of Bcl-2 (by anti-Bcl-2 western blot analysis) and for GTP-binding activity (by exposing the nitrocellulose-immobilized proteins to [α-^{32}P]GTP). Bcl-2 was found exclusively in the immune pellets, and had been efficiently cleared from the supernatant fractions under these conditions (Fig. 2*b*, lower panel). By contrast, no GTP-binding proteins were detected in the immune pellets (Fig. 2*b*, upper panel) as they remained completely in the supernatant fractions. These results showed that, using highly specific anti-Bcl-2 antibodies, Bcl-2 can be completely dissociated from the small M_r G proteins present in B lymphocytes, further suggesting that Bcl-2 does not bind GTP under the conditions used.

It is unlikely that the lack of observed GTP-binding by Bcl-2 immunoprecipitates (which contrasts with results reported earlier[16]) resulted from inactivation of the protein, as the GTP-binding activity of the small M_r G proteins remained intact following incubation with our antiserum. Furthermore, Ras immunoprecipitated from FL18 cells with the Ras11 antibody could renature to bind GTP under our experimental conditions (unpublished observations). Low levels of GTP-binding by Bcl-2 in our immunoprecipitates were ruled out by prolonged overexposure of the autoradiographs (data not shown). Finally, a recombinant Bcl-2 protein overexpressed at high levels in *Escherichia coli*[10] failed to bind GTP in conditions in which the recombinant Ras protein binds[21].

Bcl-2 has a predicted M_r of 26K but migrates in SDS-PAGE with a lower apparent M_r of ~24K (ref. 10). So it seems to co-migrate in SDS-PAGE with an ubiquitous small M_r G protein of B cells. We found electrophoretic conditions (Fig. 3*a*), however, in which the Bcl-2-reactive band on western blot analysis migrated in a slightly different position from this non-Bcl-2 small M_r G protein. Whereas the apparent M_r of Bcl-2 is 24K, the apparent M_r of the co-migrating GTP-binding protein was estimated to be 23.5K.

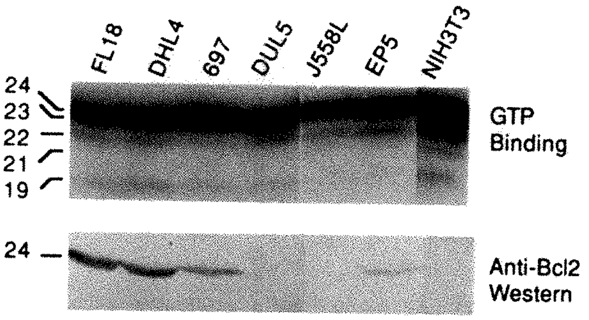

FIG. 1 Expression of Bcl-2 and low M_r GTP-binding proteins in various lymphoid and nonlymphoid cell lines. Upper panel, binding of [α-^{32}P]GTP to proteins from total cellular extracts. Lower panel, western blot of total cellular proteins using anti-Bcl-2 antibodies. Cell extracts were prepared from human B-cell lymphoma cell lines FL18, DHL4 and DUL5; human pre-B leukaemia cell line 697; mouse lymphoid cell line J558L and its Bcl-2-transfected counterpart EP5; and nontransfected NIH 3T3 cells. M_r values were determined by comparison with prestained protein standards electrophoresed in the gels.

METHODS. Cells were lysed over 30 min in buffer containing 50 mM HEPES, 150 mM NaCl, 0.5% Triton X-100, and 1 mM PMSF. Cell lysates were centrifuged at 1,000 r.p.m. for 6 min at 4 °C and extracts (5×10^6 cell equivalents per lane) prepared for 11.5% SDS-PAGE by boiling 5 min in sample buffer (0.1 M Tris-HCl, pH 6.8, 1% SDS, 0.1 M DTT). Proteins were electro-transfered to nitrocellulose using transfer buffer (50 mM Tris-HCl pH 7.4, 40 mM glycine, 1 mM SDS, 20% methanol). For GTP binding the membrane was incubated overnight at 4 °C (or 30 min at 25 °C) in binding buffer (50 mM Tris-HCl, pH 7.4, 5 mM $MgCl_2$, 0.3% Tween-20, 0.5 mM EDTA) followed by 1-h incubation at 37 °C (or 25 °C) in binding buffer plus 0.05 mCi [α-^{32}P]GTP (10 mCi ml^{-1}, Amersham 3,000 Ci mmol^{-1}). The blot was washed three times (5 min each) in binding buffer at 4 °C (or 25 °C), dryed, and autoradiographed. The membrane was then subjected to western blot analysis as previously described using anti-Bcl-2 antibodies[10].

In addition, cellular fractionation studies showed that the small GTP-binding proteins are distributed differently among various subcellular fractions compared with Bcl-2. The 23.5K and 23K small M_r G proteins were partly soluble and equally distributed between the cytosol and membrane fractions (Fig 3*b*, upper). Bcl-2, on the other hand, was not present in the cytosol and was distributed between the nuclear and membrane fractions (Fig. 3*b*, lower), consistent with its properties as an insoluble membrane-bound protein predominantly localized to the rough endoplasmic reticulum and the nuclear envelope[10].

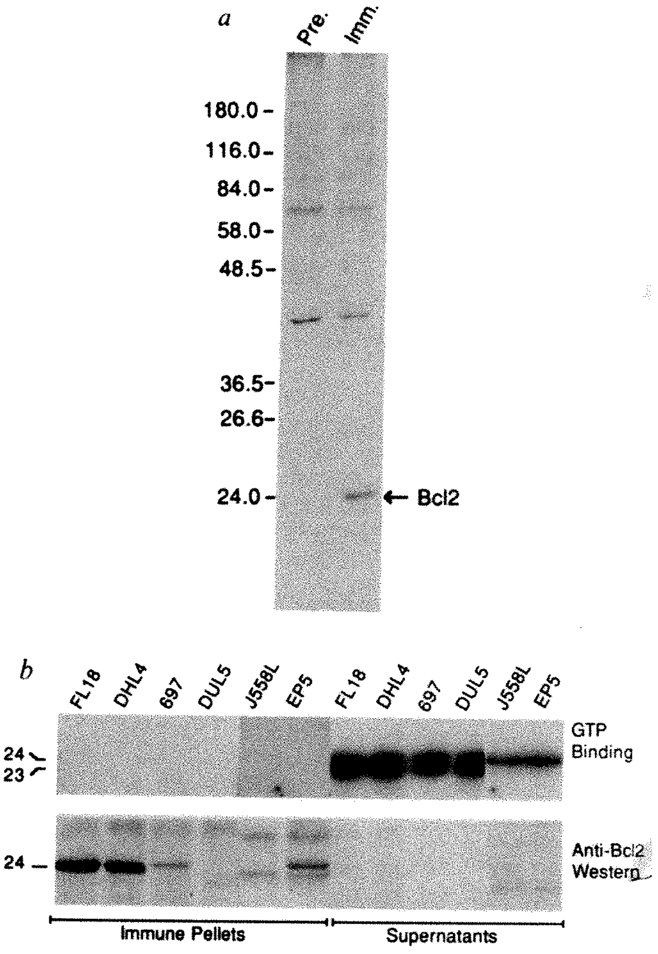

FIG. 2 Bcl-2 immunoprecipitation and GTP-binding. *a*, Immunoprecipitation of Bcl-2 from metabolically labelled FL18 cells. Pre., immunoprecipitation performed using pre-immune rabbit serum; Imm., immunoprecipitation with anti-Bcl-2 immune serum. *b*, GTP-binding (upper panel) and anti-Bcl-2 western blot analysis (lower panel) of proteins precipitated (Immune pellets) or remaining in the supernatant fraction (Supernatants). M_r standards were those described in Fig. 1.

METHODS. *a*, FL18 cells were metabolically labelled with [^{35}S]methionine for 6 h in methionine-free DME medium (15 mCi ml^{-1}, Amersham >1,000 Ci mmol^{-1}) and washed twice in cold PBS. Cell extracts were prepared as in Fig. 1 and immune complexes were formed overnight at 4 °C by incubating cell lysates with rabbit polyclonal serum highly specific for Bcl-2 as described elsewhere[10,30]. Immune complexes were collected on Sepharose protein-A beads (Pharmacia) at 4 °C for 2 h. Immune pellets were washed sequentially with two SDS washes (Fig. 1 lysis buffer with 0.1% SDS and 0.1% Triton X-100), one NaCl wash (lysis buffer with 1 M NaCl and 0.1% Triton X-100), and one SDS wash, and prepared as in Fig. 1 for 10% SDS-PAGE. *b*, Immunoprecipitation and sample preparation of unlabelled cellular proteins was carried out as described above; immune pellets and corresponding supernatant fractions were electrophoresed on 11.5% SDS-PAGE gels except for J558L and EP5 which were electrophoresed on 7.5–11.5% gradient SDS-PAGE gels. Proteins were transferred to nitrocellulose filters and subjected to GTP-binding analysis and anti-Bcl-2 immunoblotting as described in Fig. 1.

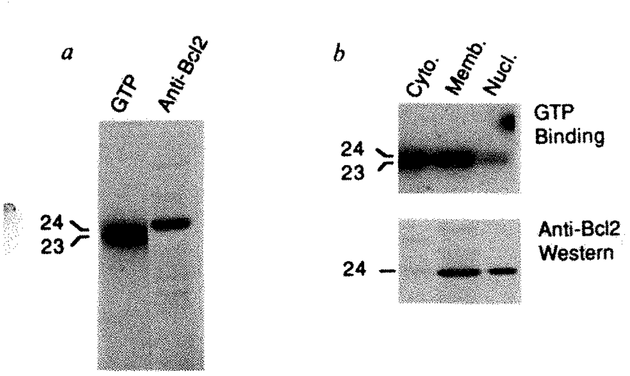

FIG. 3 Physical and biochemical fractionation of Bcl-2 and small M_r G proteins in lymphoid cells. *a*, Migration differences of Bcl-2 and small M_r GTP-binding proteins on SDS-PAGE gels. Total cellular proteins were fractionated by 11.5% SDS-PAGE and a single gel lane was subjected sequentially to GTP-binding (lane GTP) and anti-Bcl-2 western blot (anti-Bcl-2) analyses. Western filter and corresponding autoradiograph were aligned to show small but reproducible differences in migration for Bcl-2 and small M_r G proteins of 24K. *b*, Cellular fractionation demonstrating subcellular distributions of Bcl-2 and small M_r G proteins. Upper panel, GTP-binding analysis; lower panel, subsequent western blot analysis of the same filter. Cyto., soluble cytoplasmic fraction; Memb., cytoplasmic membrane fraction; Nucl., nuclear fraction. METHODS. *a*, FL18 cell extract was prepared as described (Fig. 1). *b*, Cell fractionation was performed as previously described[10]. Samples were run on 11.5% SDS-PAGE and subjected to GTP-binding and anti-Bcl-2 western blot analyses (Fig. 1, methods).

The above observations indicate that Bcl-2 is not one of the small M_r G proteins expressed ubiquitously in the lymphoid cell lines investigated here. The possibility that Bcl-2 binds GTP under other conditions seems unlikely as we have been unable to detect significant binding of GTP in Bcl-2 immune pellets that have not been subjected to denaturing conditions (unpublished observations). These results are consistent with the absence of significant sequence similarity of Bcl-2 with GTP-binding proteins, particularly in the highly conserved region of the 'phosphate-binding' consensus sequence (Gly-X-X-X-X-Gly-Lys) (ref. 22). Previous study of potential GTP binding by Bcl-2 (ref. 16) did not account for the ubiquitous small-M_r G proteins and may have been hampered by the reduced specificity of anti-peptide antibodies as reported by others[23]. The small M_r G proteins that we have observed in B lymphocytes may correspond to those described previously in other haematolymphoid cells. The 23.5K and 23K proteins seem similar to those in platelets and neutrophils[17-18], and one may correspond to the *ral* gene product[24]. The 19K GTP-binding protein most probably is Rho (ref. 25). A 22K GTP-binding protein has also been found in platelets and probably represents the protein variously called Smg21, Krev or Rap1[26-29]. As none of the small M_r GTP-binding proteins in B lymphocytes corresponds to Bcl-2, the biochemical role of this proto-oncogenic protein remains to be determined. ☐

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The c-*ets* proto-oncogenes encode transcription factors that cooperate with c-Fos and c-Jun for transcriptional activation

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CELL transformation by oncogenes leads to changes in gene expression. A key event in this process seems to be activation of the transcription factors AP-1 and PEA 3 (refs 1–5). Their synergistic activities are required for efficient activation of transcription from different promoters by many different oncogenes, serum growth factors and the tumour promoter TPA[4,5]. We show here that the products of the *ets*-1 and -2 proto-oncogenes, whose biological function was previously unknown[6–11], are transcription factors that activate transcription through the PEA 3 motif. The p68$^{c\text{-}ets\text{-}1}$ protein specifically binds to DNA and contains a transcriptional activation domain. The *ets*-like gene family therefore seems to encode a new family of transcription factors, apparently unrelated to other transcription factors. The p68$^{c\text{-}ets\text{-}1}$ protein cooperates with c-Fos and c-Jun (components of AP-1) for activation of transcription from the oncogene-responsive domain of the polyoma enhancer, indicating that combined activity of all three oncoproteins could be involved in the response of cells to growth stimuli.

A 14-base pair (bp) sequence from the polyoma (Py) enhancer (Fig. 1, oncogene-responsive domain) mediates transcriptional activation by serum growth factors, the tumour promoter TPA (12-*O*-tetradecanoylphorbol-13-acetate) and several oncogenes[4]. We found that expression of p68$^{c\text{-}ets\text{-}1}$ protein in LMTK$^-$ fibroblasts efficiently activated transcription through this 14-bp sequence (~20-fold; Fig. 1*b*, lanes 2, 3, 5, 6, 10, 12). Mutations in the PEA 3 motif of this element abolished p68$^{c\text{-}ets\text{-}1}$ activation (four tandem copies of sequences with mutations M1, M3 or M6; Fig. 1*c*, lanes 2–4, 9–11). Mutations in the AP-1 motif did not hamper efficient activation (four tandem copies of sequences with mutations M7, M4 or M5; Fig. 1*c*, lanes 5–7, 12–14). Expression of p68$^{c\text{-}ets\text{-}1}$ activated transcription through a PEA 3 motif multimer (~10-fold; Fig. 1*b*, lanes 7, 10, 9, 12), demonstrating that the PEA 3 motif is sufficient for transactivation by p68$^{c\text{-}ets\text{-}1}$.

To investigate whether p68$^{c\text{-}ets\text{-}1}$ binds to the PEA 3 motif, p68$^{c\text{-}ets\text{-}1}$ was synthesized in rabbit reticulocyte lysates and used for gel-retardation experiments with a PEA 3 oligonucleotide probe. A retarded complex that was absent from control extracts was observed (Fig. 2, lanes 1, 3, 4). No such complex was observed when an M1-mutated PEA 3 probe was used (lanes 5, 7). This mutation prevents transactivation by p68$^{c\text{-}ets\text{-}1}$ (Fig. 1*c*). Competition experiments showed that the wild-type sequence was the better competitor (Fig. 2, compare lanes 12-15 with 8-11). These results show that p68$^{c\text{-}ets\text{-}1}$ specifically binds to the PEA 3 motif.

Transcription factors contain activation domains that can stimulate transcription when fused to a heterologous DNA-binding domain. The p68$^{c\text{-}ets\text{-}1}$ protein fused to the DNA-binding domain of the bacterial repressor LexA (LexA-ets68) activated transcription through the *lexA* operator (Fig. 3, lanes 3, 7). Expression of either LexA alone, or unfused LexA with p68$^{c\text{-}ets\text{-}1}$, had no effect on transcription (Fig. 3, lanes 1, 2, 5, 6). Coexpression of inactive LexA inhibited LexA-ets68 (Fig. 3, lanes 3, 4, 7, 8), indicating that LexA competes with LexA-ets68 for binding to the operator and thereby inhibits its activity. These results are consistent with the presence of one or more transcriptional activation domains in p68$^{c\text{-}ets\text{-}1}$.

We previously suggested that there are cooperative interactions between PEA 3 and AP-1 (ref. 4). We tested whether p68$^{c\text{-}ets\text{-}1}$ could complement AP-1 (c-Fos and c-Jun) to activate transcription from the Py oncogene-responsive domain. Expression in LMTK$^-$ cells of limiting amounts of p68$^{c\text{-}ets\text{-}1}$ or c-Fos and c-Jun proteins led to low levels of activation of the reporter recombinant containing four copies of the B sequence (4- and 20-fold respectively, compared with the effects on the pG1 control reporter; Fig. 4, lanes 5-7 and 1-3). By contrast, coexpression of p68$^{c\text{-}ets\text{-}1}$ with AP-1 led to a much greater activation (250-fold; Fig. 4, lanes 4 and 8). It is of interest that a monomer B sequence is not detectably activated by either p68$^{c\text{-}ets\text{-}1}$ or AP-1 expression, and their combined effect is required for efficient activation (results not shown). Both the c-Ets-1 and AP-1 binding sites are required for cooperativity, as p68$^{c\text{-}ets\text{-}1}$ and AP-1 activate only through their corresponding motifs (ref. 4 and Fig. 1*c*). There was no complementation when only one of the motifs (PEA 3 or AP-1) was present in the reporter (results not shown). Mutations in part of either motif in the oncogene-responsive domain diminished activation by combined expression of p68$^{c\text{-}ets\text{-}1}$ and AP-1, but to a lesser extent than would be expected from the effect of the mutation on activation by one of the factors alone (results not shown). These data show that p68$^{c\text{-}ets\text{-}1}$ can cooperate with AP-1 in transcriptional activation. Expression of p68$^{c\text{-}ets\text{-}1}$ inhibits basal-level transcription from the oncogene-responsive domain when its own binding site is mutated (0.1-, 0.2- and 0.2-fold for M1, M3 and M6 respectively), but only when the AP-1 site is present

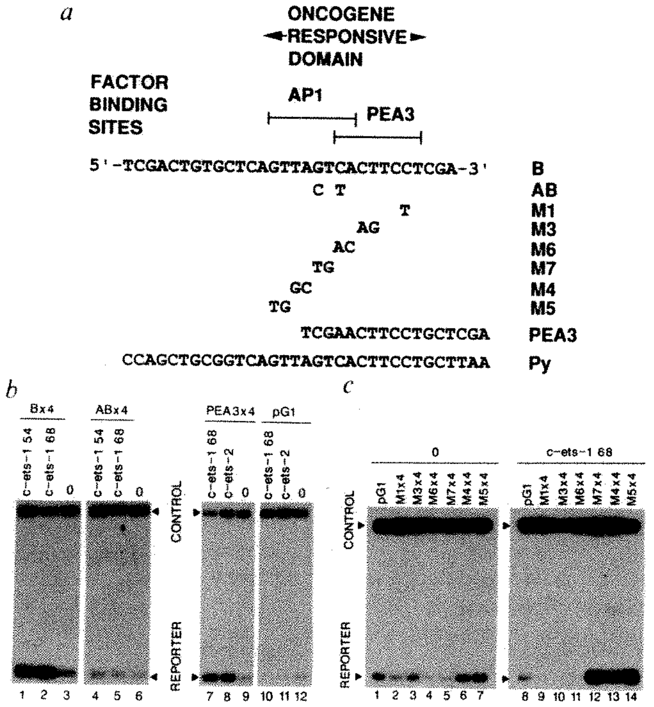

FIG. 1 *a*, Sequence of transcription elements. Transcriptional activation by the oncogene-responsive domain is measured by the ability of the transcription elements to stimulate transcription from the β-globin gene promoter in the reporter recombinant gene pG1 (ref. 4). The inserts are either four tandem copies of mutants B, AB or M1-M7 (B×4, AB×4, M1-M7×4) or one copy of the sequences shown. The oncogene-responsive domain of the Py enhancer contains specific binding sites for the transcription factors AP-1 and PEA 3. The extent of the PEA 3 site in the B sequence is deduced from the ability of the wild-type and mutated sequences to specifically mediate PEA 3 binding. The PEA 3 oligonucleotide contains the PEA 3 motif as defined by Martin *et al.*[23]. This sequence contains several additional nucleotides from the Py enhancer which compensate functionally for the loss of the C nucleotide that is mutated in the AB oligonucleotide (data not shown, see text). *b*, Activation of transcription by members of the *ets* gene family. LMTK$^-$ mouse fibroblasts were transfected with 1 μg reporter recombinants (inserts B×4, AB×4, PEA 3×4 or none (pG1)), 0.5 μg internal control plasmid (pβCB×2, ref. 4), and 0.5 μg expression vectors for p68$^{c\text{-}ets\text{-}1}$, p54$^{c\text{-}ets\text{-}1}$ or p58-64$^{c\text{-}ets\text{-}2}$. *c*, p68$^{c\text{-}ets\text{-}1}$ activates transcription through the PEA 3 motif. LMTK$^-$ fibroblasts were transfected with 1 μg reporter recombinants (inserts 4×M1, M3, M6, M7, M4 or M5, or none (pG1)), 0.5 μg internal control (pβCB×2) and 0.5 μg c-Ets-1 68 expression vector (lanes 8-14) or the expression vector pSG5 without an insert (lanes 1-7). Reporter and control indicate the corresponding specific S1 nuclease-resistant products.

METHODS. LMTK$^-$ cells were transfected with the indicated recombinants by the DEAE dextran and chloroquine technique. The cells were incubated in low-concentration serum for 48 h, then total RNA extracted and analysed by quantitative S1 nuclease mapping for RNAs specifically initiated from the β-globin gene promoter of the reporter recombinant and the conalbumin gene promoter of the internal co-transfected control recombinant[4]. The expression vectors contain the corresponding *c-ets* complementary DNA sequences in the multiple cloning site of the pSG5 expression vector[24].

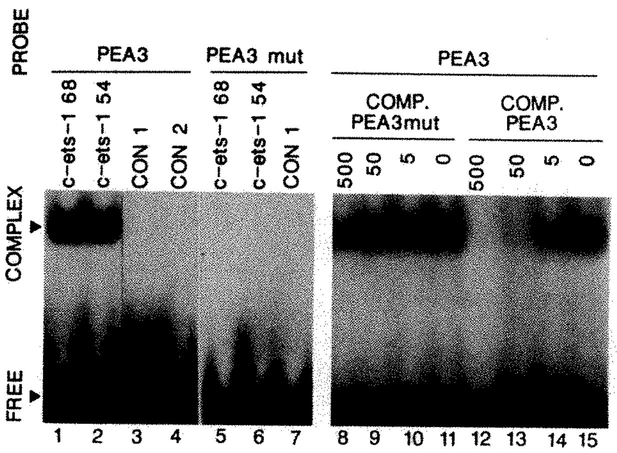

FIG. 2 *In vitro*-synthesized c-Ets-1 specifically binds to the PEA 3 motif. The p68$^{c\text{-}ets\text{-}1}$ and p54$^{c\text{-}ets\text{-}1}$ proteins were translated in rabbit reticulocyte lysates (Amersham; as described in the manufacturer's instructions) from RNA transcribed by T7 polymerase from linearized pSG5 expression vectors[24] containing cDNAs for c-Ets-1 68 (lanes 1, 5, 8-15), c-Ets-1 54 (lanes 2, 6), or no insert (CON1, lanes 3, 7). Control experiments showed that full-length c-Ets-1 proteins were efficiently synthesized in these lysates. Aliquots (2 μl) were used for gel-retardation assays with either wild-type (lanes 1-4, 8-15) or mutated (M1 mutation, lanes 5-7) end-labelled PEA 3 probes as described previously[4]. For competition assays (lanes 8-15), the indicated molar excess of cold probe was incubated for 15 min at 0 °C with the *in vitro*-synthesized proteins before the addition of labelled probe. CON1, RNA synthesized from vector without insert; CON2, lysate without added RNA; COMPLEX, specific retarded complex; FREE, excess unretarded probe; COMP, competitor. Similar results were obtained with lysates primed with RNA transcribed by SP6 from different promoter-template combinations (data not shown).

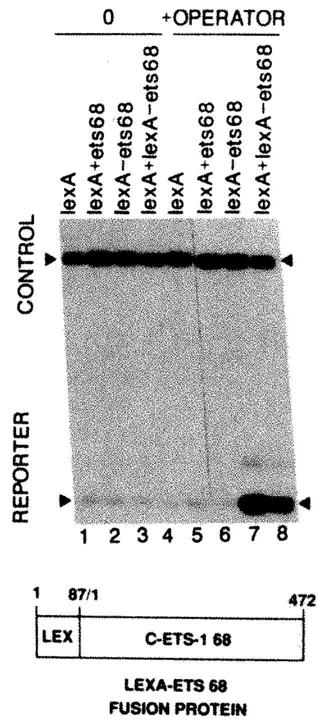

FIG. 3 The p68$^{c\text{-}ets\text{-}1}$ protein activates transcription when fused to the LexA DNA-binding domain through the *lexA* operator. LMTK$^-$ cells were transfected with 1 μg reporter recombinant containing either the *lexA* operator (lanes 5–8) or no insert (lanes 1–4), 0.5 μg internal control (pβCB × 2, ref. 4) and 1 μg expression vectors that encode either a LexA DNA-binding domain c-Ets-1 68 fusion protein (lanes 3, 7; the expression vector codes for a fusion protein containing LexA amino acids 1–87 and c-Ets-1 amino acids 1–472; bottom), or the LexA DNA-binding domain alone (lanes 1, 5), or separately for the LexA DNA-binding domain and p68$^{c\text{-}ets\text{-}1}$ (lanes 2, 6), or separately for the LexA DNA-binding domain and the LexA-Ets fusion protein (lanes 4, 8).

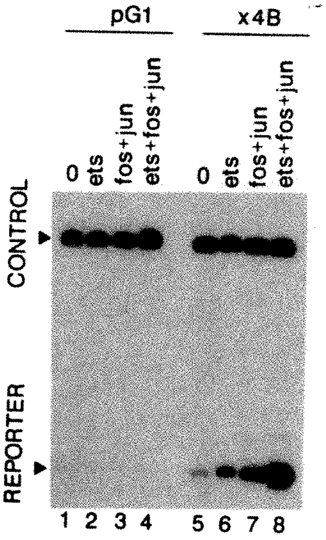

FIG. 4 Complementation between p68$^{c\text{-}ets\text{-}1}$ and c-Fos and c-Jun (AP-1) for transcriptional activation through the oncogene-responsive domain. LMTK$^-$ fibroblasts were transfected in low serum concentrations (0.05% FCS) with 1 μg reporter recombinants containing either four copies of the B mutant (lanes 5–8) or no (lanes 1–4) insert, 0.5 μg internal control (pβCB × 2) and 0.1 μg expression vectors for c-Ets-1 68, c-Fos or c-Jun[4] as indicated. Total RNA was analysed by quantitative S1 nuclease analysis. Reporter and control indicate the corresponding specific bands.

(see pG1, Fig. 1*c*, lanes 1 and 8). Similar results were obtained with a monomeric oncogene-responsive domain (data not shown). These observations could be explained by a squelching-type mechanism, in which p68$^{c\text{-}ets\text{-}1}$ specifically binds to AP-1; but other mechanisms cannot be excluded at present.

Expression of p54$^{c\text{-}ets\text{-}1}$, which differs from p68$^{c\text{-}ets\text{-}1}$ at its N terminus[10], activated transcription through the PEA 3 motif (Fig. 1*b*, lanes 1 and 4, and results not shown). The p54$^{c\text{-}ets\text{-}1}$ protein specifically bound to the PEA 3 motif (Fig. 2, lanes 2, 6, and data not shown). Therefore, the sequence differences between p68 and p54 do not significantly affect their ability to activate transcription in the systems tested.

Expression of p58-64$^{c\text{-}ets\text{-}2}$, another member of the *ets* gene family[11], activated transcription through the PEA 3 motif to a similar extent as expression of p68$^{c\text{-}ets\text{-}1}$ (Fig. 1*b*, lanes 8, 9 and 11, 12). These results indicate that the domains conserved between c-Ets-1 and c-Ets-2 are required for transcriptional activation.

The c-Ets proteins have DNA-binding and transcriptional activation domains. It seems likely that the DNA-binding domain is located in the C-terminal part of the protein that is basic, highly conserved between different *ets* gene family members[10–15] and involved in nonspecific DNA-interactions[16–20]. DNA-binding domains are highly conserved in other families of transcription factors[3,21]. Preliminary evidence indicates that the N terminus contains an activation domain (data not shown). There is no obvious homology between the *ets* family and other transcriptional activators, indicating that this is a new family of transcription factors. The oncogene-responsive domain of the Py enhancer and collagenase promoter are functionally equivalent to the type II proto-enhancer, where the association of two different motifs, which are weakly active on their own, generates a proto-enhancer, the building block of enhancers[22]. The oncogene-responsive domain represents a particular form of the type II proto-enhancer in that its activity is regulated by mitogens (and many oncogens) and it contains the motifs for three oncoprotein transcription factors. It is of interest that associated PEA 3 and AP-1 motifs are found in several oncogene-responsive promoters of the genes for stromelysin, interleukin-2, Fos and JE (see ref. 4 for discussion). The importance of *ets* and *fos* and *jun* in mediating transcriptional activation and transformation by non-nuclear oncogenes remains to be established. ☐

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The candidate Wilms' tumour gene is involved in genitourinary development

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WILMS' tumour is an embryonic kidney tumour thought to arise through aberrant mesenchymal stem cell differentiation[1] and to result from loss of function of a 'tumour suppressor' gene(s)[2]. Both sporadic and syndrome-associated Wilms' tumours are accompanied by an increased frequency of abnormalities of the urinary tract and genitalia[3]. Deletional analysis of individuals with the WAGR syndrome[4-8] (for, Wilms' tumour, aniridia, genitourinary abnormalities and mental retardation) showed that a Wilms' tumour gene lies at chromosomal position 11p13. This led to the isolation of a candidate Wilms' tumour gene[9,10], encoding a zinc-finger protein which is likely to be a transcription factor. To gain insight into the role of this candidate gene in normal development and tumorigenesis, we have now performed *in situ* messenger RNA hybridization on sections of human embryos and Wilms' tumours. The candidate Wilms' tumour gene is expressed specifically in the condensed mesenchyme, renal vesicle and glomerular epithelium of the developing kidney, in the related mesonephric glomeruli and in cells approximating these structures in tumours. The other main sites of expression are the genital ridge, fetal gonad and mesothelium. These data suggest that (1) this candidate is indeed a Wilms' tumour gene, (2) the associated genital abnormalities are pleiotropic effects of mutation in the Wilms' tumour gene itself, in support of recent genetic analysis[11], and (3) this gene has a specific role in kidney development and a wider role in mesenchymal–epithelial transitions.

We have investigated the expression of the candidate Wilms' tumour gene by northern blot analysis and by *in situ* hybridization. For this we used the complementary DNA probe *WT2-1*, known to detect a transcript of ~3 kilobases (kb) in fetal kidney and spleen[9]. Northern analysis (Fig. 1) showed that this probe detected a 3.2-kb transcript not only in fetal kidney and spleen but also in fetal testis and ovary, with an additional 2.7-kb transcript detectable in fetal testis. There was very weak expression in fetal brain. No expression was detected in fetal heart, skin, adrenal, stomach, liver, eye or muscle.

In situ mRNA hybridization was first carried out on 18-week-gestation human kidney, chosen because it includes all stages of nephron development from uninduced blast cells at the periphery to functioning nephrons towards the centre. This full range of stages is seen because the process of kidney development, involving a reciprocal inductive interaction between the ureteric bud and the metanephric blastema, is continuous from 6-34-weeks gestation[12,13]. If the candidate gene (*WT2-1*) is involved in the initiation of Wilms' tumour, then, to explain tumour histogenesis we should expect to find it expressed in the multipotent blast cells of the kidney.

The *WT2-1* gene was expressed in a very specific manner within the developing nephron (Fig. 2*a*). Expression was weak in the condensing blastemal cells, stronger in the renal vesicle—an epithelial structure arising from the condensate—and then restricted to the maturing renal corpuscle. Here, expression was particularly strong in the developing podocyte cells of the glomerular epithelium, but, as the corpuscle matured, expression declined. By contrast, no expression could be seen in the remainder of the nephron (the proximal and distal tubules and loops of Henlé) or in the derivatives of the ureteric bud (the ureter and collecting tubules). A similar but much less intense pattern of glomerular labelling was seen in normal kidney adjacent to a Wilms' tumour removed from a 10-month-old male (data not shown).

To investigate whether the gene has a role in earlier development, we examined sections of 6-7-week human embryos by *in situ* mRNA hybridization (Fig. 2*b*). Here, *WT2-1* expression in the metanephric blastema matched that in the 18-week kidney; it was very strong in the condensing blast cells, but absent in the uninduced mesenchyme and ureteric bud branches. The *WT2-1* gene was also expressed in the glomerular epithelium of the related mesonephros and in the developing gonad, mainly over the germinal epithelium and the sex cords, but not over those germ cells that could be identified morphologically. Of the remaining tissues that could be seen clearly, expression was restricted to the mesothelial lining of the coelomic cavity and its contents, with some expression in the somatopleuric mesoderm immediately underlying this layer dorsolaterally.

If *WT2-1* plays a primary role in Wilms' tumour, we would expect mutations affecting this gene to be present in a significant number of tumours, although perhaps not in all, as more than one locus has been implicated in the disease[14-17]. We analysed 13 tumours by northern blot (Fig. 3) and found that each expressed the 3.2-kb transcript. This result is not unexpected in the light of retinoblastoma observations, where most fresh

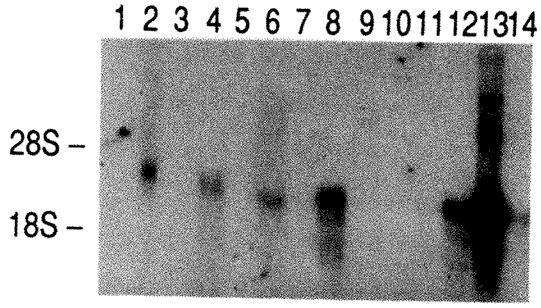

FIG. 1 Northern blot analysis of *WT2-1* expression in human fetal tissues. Lanes: 1, adrenal; 2, kidney; 3, heart; 4, ovary; 5, skin; 6, spleen; 7, stomach; 8, testis; 9, eye; 10, liver; 11, brain; 12, HEK1; 13, kidney; 14, 293 cells. Lanes 1–7, total RNA; and 8–14, poly(A)$^+$ RNA. HEK1 is a primary culture of human fetal kidney cells. 293 is an adenovirus-transformed human embryonic kidney cell line (ATCC number: CRL 1573). A 3.2-kb transcript is present in fetal kidney, spleen, testis, ovary and brain, with an additional 2.7-kb transcript seen in testis when poly(A)$^+$ RNA is used. In this experiment the RNAs ran on a slant, but we have confirmed in independent analyses that the 3.2-kb transcript is the same size in all positive tissues. Size markers shown (left).

METHODS. Human fetal tissues were obtained at autopsy of 10–19-week gestation fetuses. RNA was prepared from frozen tissues by the guanidine isothiocyanate/cesium chloride gradient method[24]. Total RNA (20 μg) or poly(A)$^+$ RNA (10 μg) was loaded, electrophoresed through 1% agarose-formaldehyde gels and transferred to Hybond-N, as described[25]. Filters were probed with *WT2-1*, a 1.5-kb *Eco*RI-*Pst*I fragment of cDNA 2-1. This was isolated from a human fetal kidney cDNA library by screening with the conserved single copy probe J803p4, which maps to the Wilms' tumour locus at 11p13 (ref. 9). High-specific-activity ^{32}P-labelled DNA probes were prepared for northern blot hybridization using a Random Prime kit according to manufacturers' instructions (Boehringer-Mannheim). Hybridization was at 68 °C in 7% SDS, 0.5 M sodium hydrogen phosphate, 1 mM EDTA. Filters were washed at 68 °C in 2×SSC, 0.1% SDS and exposed to Kodak XAR film for 18 h. Loadings were standardized by reprobing with actin (data not shown).

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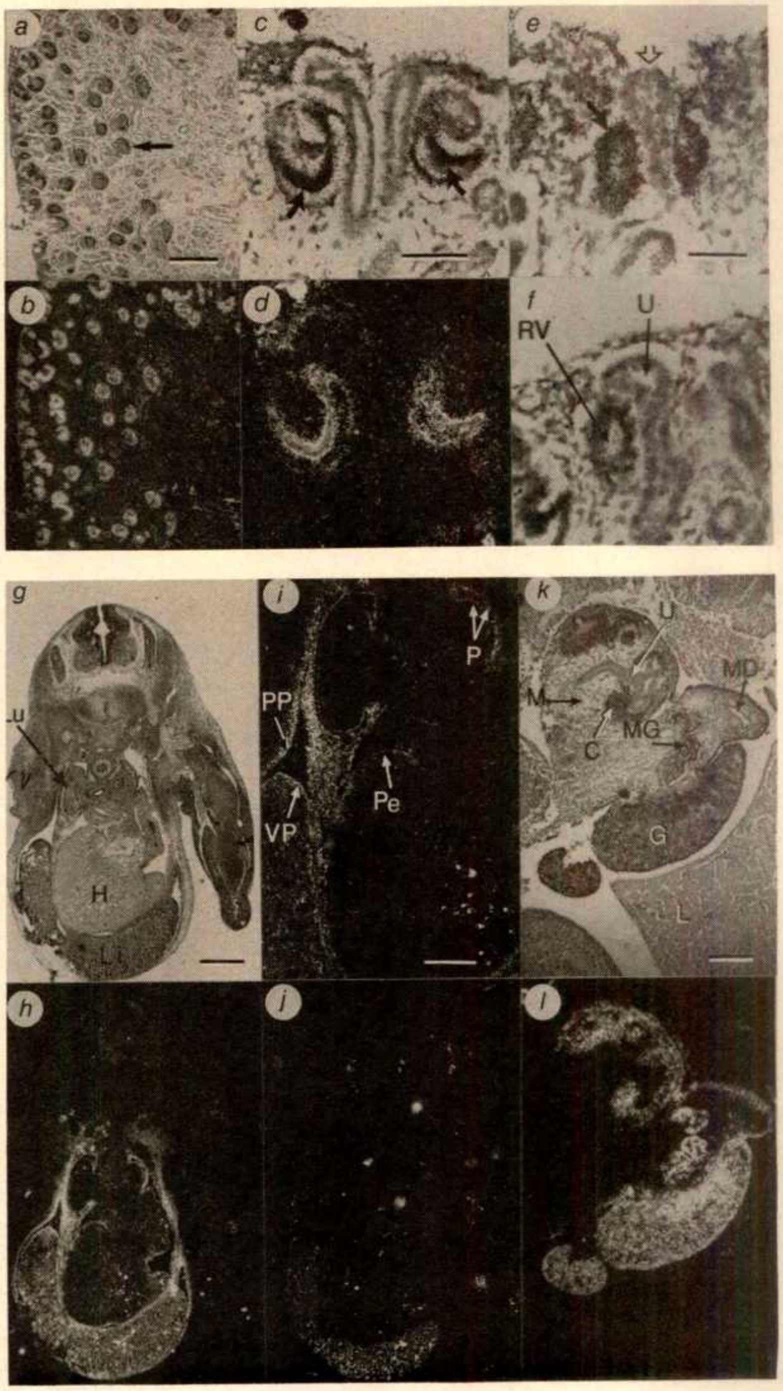

FIG. 2 *a–f*, Expression pattern of *WT2-1* in an 18-week gestation human fetal kidney as revealed by *in situ* mRNA hybridization. All stages of nephrogenesis (see refs 12 and 13 for reviews) are shown. The active nephrogenic zone is most peripheral. *a*, Low power view of longitudinal section of kidney, hybridized with antisense probe. Labelling is restricted to the subcortical condensed blastema, renal vesicle and podocyte layer of the renal corpuscle. More mature corpuscles (arrow) lie farthest from the cortex and label less strongly. Bar, 1 mm. *c*, High power view of *a* showing labelling confined to the presumptive podocyte layers (arrow) of the S-shaped body (right) and immature glomerulus (left). Bar, 200 μm. *b* and *d*, Dark-field view of *a* and *c*, respectively. *e*, High-power view of *a* showing uniformly labelled renal vesicle (arrow) adjacent to its inducing ureteric bud ampulla (hollow arrow), which does not label. Bar, 100 μm. *f*, Similar structure to *e* from adjacent section of same kidney hybridized to sense (control) probe. RV, renal vesicle; U, ureteric bud ampulla. *g–l*, Expression pattern of *WT2-1* in human embryos, as revealed by *in situ* mRNA hybridization. *g*, Transverse section through upper thoracic region of a 43-day human embryo, hybridized with antisense riboprobe for *WT2-1*. Bright field reveals structure only. Lu, lung bud; H, heart; Li, liver. Bar, 0.5 mm. *h*, Same section viewed under dark field illumination to reveal silver grains showing sites of binding of *WT2-1*. Labelling of the liver parenchyma is nonspecific as it also occurs with the control (sense) probe. *i*, Higher-power view of *h* showing labelling of pleural, peritoneal and pericardial layers. Pe, pericardium; PP, parietal peritoneum; VP, visceral peritoneum; P indicates both pleural layers. Bar, 200 μm. *j*, Adjacent section to *g* labelled with sense *WT2-1* riboprobe. Liver binding is nonspecific. *k*, Transverse section through lower abdominal region of a 49-day gestation human embryo, labelled with antisense *WT2-1* riboprobe. Heavy labelling is seen over the gonadal ridge (G), condensed metanephric blastema (C), glomerular structures of the mesonephros (MG) and the epithelial layer lining the coelomic cavity. The mesonephric duct (MD), ureteric bud branches (U) and uncondensed metanephric mesenchyme (M) are unlabelled. An adjacent section hybridized with the sense probe showed no labelling (data not shown). Bar, 200 μm. *l*, Same section as *k* viewed under dark-field illumination.

METHODS. Fetal kidneys were obtained at autopsy and embryos were obtained following legal termination of pregnancy. Specimens were fixed in 4% paraformaldehyde and wax-embedded. Sections (7 μm) were mounted onto TESPA-coated slides. *In situ* hybridization was performed as described[26], including hybridization at 55 °C in 50% formamide, high stringency washes at 65 °C in 50% formamide and RNase A treatment. Antisense and sense (control) riboprobes for the *WT2-1* gene were prepared by [^{35}S] UTP incorporation; 1.2×10^6 d.p.m. were applied to each section. In all experiments, the sense probe was routinely included and gave no labelling above background.

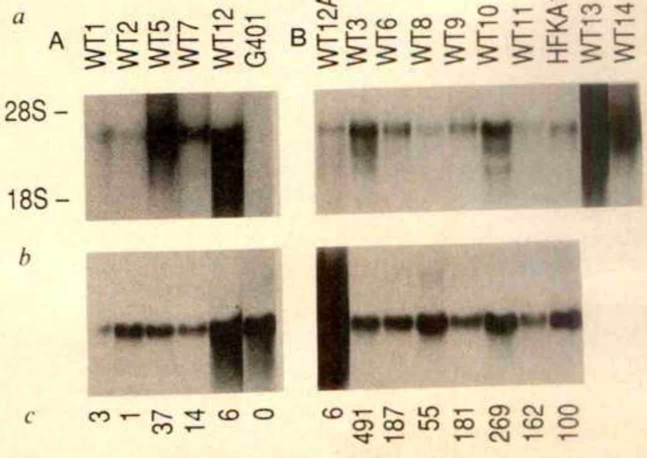

FIG. 3 Northern blot showing variation in level of *WT2-1* expression in Wilms' tumours. The same filters were hybridized to *WT2-1* (*a*) or glyceraldehyde phosphate dehydrogenase[27] (*b*) (autoradiographs were exposed for 48 h (A) and 5 h (B)). WT13 and 14 were run separately and exposed for 36 h. G401, Wilms' tumour cell line. Autoradiographs were scanned densitometrically[28] and the level of *WT2-1* expression relative to that of human fetal kidney (100%) is shown in *c*, using GAPDH to standardize loadings. Methods as in Fig. 1, but filters were washed in 0.5 × SSC.

tumour samples express a full-length transcript of the retinoblastoma gene[18], mutations only being revealed by methods detecting point mutations or small rearrangements[19]. We did, however, find an almost 500-fold variation in *WT2-1* expression, an observation that prompted us to try to correlate retrospectively expression with tumour histology. We found that tumours that were predominantly blastemal or showed epithelial differentiation expressed high levels of *WT2-1*, but tumours with stromal predominance had low levels. This correlation was confirmed directly by *in situ* mRNA hybridization analysis of seven Wilms' tumours (Fig. 4); highest levels of expression were found in tubular structures at a stage immunohistochemically equivalent to the early renal vesicle in normal development, while blastemal cells showed lower levels of expression but were clearly positive relative to the nonexpressing stromal cells. Thus, *WT2-1* expression is found only in the malignant counterparts of cell types that express the gene during normal kidney development.

Our results, taken together with the now precisely known chromosomal localization of the gene and its structural homologies[9,10], strengthen the case for *WT2-1* being a Wilms' tumour gene. The specific expression pattern of *WT2-1* contrasts with the behaviour of the retinoblastoma gene—a putative cell-cycle regulator—which is ubiquitously expressed, but gives rise through unknown mechanisms to tumours that are tissue-restricted[20]. As to the origins of Wilms' tumour, our observations lead us to the following hypothesis. During normal kidney development, the *WT2-1* gene product acts as a transcriptional regulator to switch off genes involved in maintaining cell proliferation and/or switch on genes involved in blastemal differentiation. In tumours, we suggest that the gene retains its responsiveness to its upstream regulators in the proliferative response pathway, so that it is transcribed in the appropriate cell types, but that mutation alters the ability of its product to regulate downstream target gene(s), so leading to uncontrolled proliferation with aberrant differentiation and eventual tumour formation.

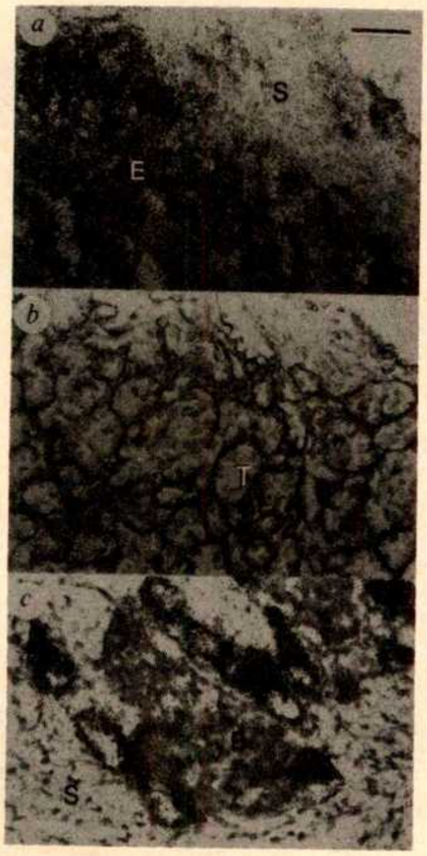

FIG. 4 Expression pattern of *WT2-1* in Wilms' tumours WT13 and 14 as shown by *in situ* mRNA hybridization. *a*, WT13 hybridized with antisense probe. There is heavy labelling over areas of epithelial differentiation (E) but none over stromal areas (S). Bar, 100 μm. *b*, Similar area in nearby section of same tumour, labelled immunocytochemically with antibodies to laminin, showing polarized basement membranes of tubular structures. Antibodies to collagen IV gave a similar result. These tubules were negative for cytokeratins 8 and 18 on an adjacent section labelled with CAM 5.2, suggesting that they are at a stage equivalent to the early renal vesicle in normal development[29,30] and not the mature tubule. *c*, WT14 hybridized with antisense probe. Labelling is seen over blastemal cells (B) and tubular structures (T) but not over the stroma (S). Similar patterns of expression were seen in five other Wilms' tumours (data not shown).

METHODS. Tumours were obtained fresh at the time of nephrectomy. Tissue fixation and *in situ* hybridization were performed as described in Fig. 2. Immunohistochemical staining was performed using polyclonal rabbit anti-laminin antibodies (Eurodiagnostics) at a 1 in 50 dilution by the peroxidase–antiperoxidase method, as described[31].

The observations presented here also cast light on other aspects of phenomena associated with normal and abnormal kidney development. The fact that the gene is expressed in both the podocytes and the more primitive mesonephros argues for it playing an important part in the development of the specialized filtration function of the kidney, a role that may have evolved early in vertebrate evolution. More intriguing, perhaps, is the observation that the gene is expressed in three tissues undergoing the rare transition from mesenchyme to epithelium: the differentiation of the metanephric mesenchyme to nephrons, the formation of the mesothelium from the mesenchyme lining the coelom and the production of sex cords from the mesenchyme of the primitive gonad. It will be interesting to investigate whether tumours from these tissues express the *WT2-1* gene.

The expression of *WT2-1* in the developing gonad gives a molecular basis for several phenomena seen in individuals with Wilms' tumour. First, the genital abnormalities seen as part of the WAGR syndrome and associated with sporadic Wilms' tumour could be pleiotropic effects of mutation in the Wilms' tumour gene. Second, both the pseudohermaphroditism and nephropathy seen as part of the Wilms' tumour-associated Drash syndrome[21] could be accounted for by disruption of a single gene. Finally, the lack of inheritance of Wilms' tumour-predisposing mutations at the 11p13 locus[14,15] could be due to such mutations causing infertility. This speculation arises from our observation of *WT2-1* expression in non-germ-cell components of the fetal gonad, from which develop the epithelial components of both testis and ovary. In abnormalities of gonadal epithelial development, both gametogenesis and the hormone-dependent development of the external genitalia may be faulty[22]. Thus, both infertility and anatomical abnormalities of the genitalia could result from mutation or reduced dosage of this gene, perhaps in a manner analogous to that described for testis determination.[23]. □

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Analysis of gain-of-function mutations of the *lin-12* gene of *Caenorhabditis elegans*

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CERTAIN cell fate decisions are specified by cell–cell interactions during the development of the nematode *Caenorhabditis elegans*. For example, in a wild-type hermaphrodite gonad, two cells, Z1.ppp and Z4.aaa, have the potential to become the anchor cell (AC)[1]. Intercellular communication establishes their fates and ensures that only one cell becomes the AC, while the other becomes a ventral uterine precursor cell (VU)[2,3]. One component of this intercellular communication seems to be the 'AC-to-VU' signal from the presumptive AC that causes the other cell to become a VU[3]. Genetic and developmental studies[3,4] indicate that the *lin-12* gene specifies the fates of Z1.ppp and Z4.aaa. Molecular studies[5,6] suggest that *lin-12* directly participates in their communications, perhaps acting as the receptor for the 'AC-to-VU' signal[3]. Here, we report the molecular lesions associated with *lin-12* gain-of-function mutations, cell isolation experiments, and genetic studies of an unusual *lin-12* allele. These data suggest that self-association of the putative *lin-12*-encoded receptor leads to its activation, and that certain gain-of-function mutations result in ligand-independent activation.

Whereas *lin-12(0)* mutations are recessive and eliminate *lin-12* activity, causing both Z1.ppp and Z4.aaa to become ACs, *lin-12(d)* mutations are semidominant and cause both Z1.ppp and Z4.aaa to become VUs[4]: By genetic criteria, *lin-12(d)* mutations behave like hypermorphic (elevated activity) mutations[7]: addition of *lin-12(+)* alleles to a *lin-12(d)* mutation increases the proportion of hermaphrodites that show the transformation of the presumptive AC into a VU. So elevated *lin-12* activity is sufficient to cause expression of the VU fate. To explore how *lin-12(d)* mutations elevate *lin-12* activity, we identified the molecular lesions associated with *lin-12(d)* mutations. We analysed a 13.8 kilobase (kb) genomic region comprising the coding and flanking regions by denaturation gradient gel electrophoresis[8,9] of DNA amplified by the polymerase chain reaction (PCR; see Fig. 1 legend), and direct sequencing[10]. All of the *lin-12(d)* mutations are missense alterations of the putative extracellular region of the protein (Fig. 1). Five different mutations alter the extracellular cysteine-poor region of the protein; two others alter one of the adjacent cysteine-rich motifs (see Fig. 1). Although the mutations have nothing obvious in common, it is curious that the *n137*, *n177* and *n950*, the three 'strongest' alleles by gene-dosage criteria[4] (see Table 2), are located within four codons of one another. Because all eight *lin-12(d)* mutations alter the predicted protein sequence, they are likely to be modifying protein activity or stability.

To examine further how *lin-12(d)* mutations elevate *lin-12* activity, we performed laser ablation experiments on *lin-12(d)* mutants. In wild type, when all other somatic gonadal cells have been ablated, an isolated Z1.ppp or Z4.aaa becomes an AC. Expression of the VU fate by either cell requires both cells to be present, as one cell must send the 'AC-to-VU' signal to promote expression of the VU fate by the other cell[2,3]. In

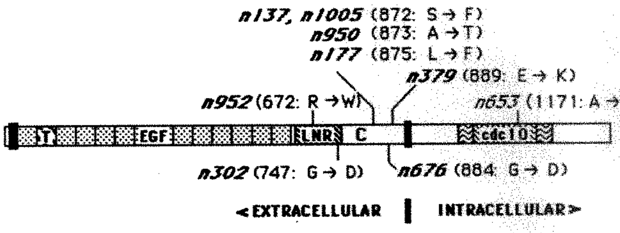

FIG. 1 Sequence alterations associated with eight *lin-12(d)* mutations and with *n653*. Predicted amino acid changes [residue number: change (single letter code)] deduced from sequence analysis of different *lin-12(d)* alleles (bold) and of the *n653* mutation, shown on a schematic depiction of the *lin-12* product[6]. EGF, EGF (epidermal growth factor) like motifs; T, 'T + Y'-encoded motif; LNR, lin-12–Notch repeated motif; cdc10, cdc10/SW16 motif; C, position of a pair of cysteines that is conserved in lin-12, glp-1 and Notch; black regions represent hydrophobic stretches that might serve as a signal-sequence (amino terminus) or membrane-spanning region (internal). The nucleotide changes observed were: *n952* (9,861 C → T), *n302* (10,087 G → A), *n137* and *n1005* (10,462 C → T), *n950* (10,464 G → A), *n177* (10,470 C → T), *n676* (10,498 G → A), *n379* (10,512 G → A). All *lin-12(d)* mutations arose after ethyl methanesulphonate (EMS) mutagenesis of a *lin-12(+)* chromosome (refs 4, 23, and E. Ferguson, N. Tsung, C. Desai and R. Horvitz, personal communication); they are all transitions, as is generally the case for EMS-induced mutations. The amino acids altered by *lin-12(d)* mutations are not necessarily conserved in other genes related to *lin-12*.
METHODS. PCR was used to amplify six 2–3 kb regions of DNA from the *lin-12* locus. The region analysed was 13.8 kb, and ranged from ~2.5 kb upstream of the apparent initiation codon (nucleotide position 1,848; numbering as in Genbank entry M21478) to 100 base pairs (bp) past the region encoding the 3′ end of the messenger RNA (position 13,109, which is in the next gene, *gst-1*; ref. 21). The PCR reaction mixtures were extracted with phenol-chloroform, precipitated with ethanol, and resuspended in (10 mM Tris buffer, 1 mM EDTA. Equal amounts of wild-type and *lin-12*-mutant DNA segments generated by PCR were mixed, boiled (2 min), and allowed to renature at room temperature (15 min). Restriction buffer and appropriate restriction enzymes (that would yield fragments ranging from 200–800 bp) were added, and the reactions were incubated until digestions were complete, as ascertained by agarose gels. Denaturing gradient gel electrophoresis (DGGE) was performed as described in refs 8 and 9; typically, three different digests of each heteroduplexed DNA mixture were subjected to DGGE for 18 h at 65V (20–80% linear gradient of denaturant, formamide and urea). Because all *lin-12(d)* mutations were generated by EMS-mutagenesis of the isogenic strain N2 (ref. 22), alterations revealed by DGGE were likely to result from the *lin-12(d)* mutations and not from strain polymorphisms. The *lin-12(d)* alleles *n379*, *n302* and *n676* were localized by DGGE to the region comprised by nucleotides 9,682–11,041; although subsequent sequence analysis revealed that the other five *lin-12(d)* mutations were located in this region, they were not detected by DGGE under the conditions that we used. This region was then amplified in all mutants and sequenced directly[10] using internal primers. No other changes were seen in any other region examined by DGGE, although not all regions were examined for every *lin-12(d)* mutation. We do not have sequence information for the region outside of 9,682–11,041, other than some spot sequencing of cloned DNAs from the region encompassed by nucleotides 11,011–13,109 (from the intracellular region of the protein) from the *lin-12(d)* alleles *n379*, *n302*, *n676*, *n952* and *n137*. The alteration associated with the *n653* mutation creates an *Rsa*I site and was recognized by agarose gel electrophoresis of *Rsa*I-digested amplified DNA from the region comprised by 11,011–13,109. The presence of the *n302* mutation in *lin-12(n302 n653)* was confirmed by sequence analysis of amplified DNA from 9,682–11,041. We examined the entire 13.8-kb region encompassing the coding and flanking regions of *lin-12(n302 n653)* by DGGE and found no other alterations or restriction site polymorphisms. The *n653* mutation results in a conservative Ala → Val change; this Ala is present in corresponding positions in both glp-1 (ref. 16) and Notch (refs 13, 14).

TABLE 1 Ablation of all gonadal cells except Z1.ppp or Z4.aaa in *lin-12(d)* mutants

lin-12 genotype[*]	*n*[†]	Fate[‡] of Z1.ppp or Z4.aaa
+	5/5[§]	AC
n379	3/3	VU
n349	4/4	VU
n676	4/4	VU
n952	7/7	VU
n137	8/8[‖]	VU

Ablations were performed as described in ref. 3.

[*] Complete genotypes: *n379* is *lin-12(n379); him-5(el1467). n952* is *unc-32(e189) lin-12(n952). n137* is *lin-12(n137); him-5(e1467).*

[†] *n*, Number of animals that exhibited the cell fate indicated divided by the total number of animals examined.

[‡] AC, anchor cell; VU, ventral uterine precursor cell. Criteria for scoring cell fates were as described[3].

[§] Data from refs 1 and 3.

[‖] Data from ref. 3.

contrast, in all five *lin-12(d)* mutants examined, an isolated Z1.ppp or Z4.aaa expresses the VU fate (Table 1)[3]. These results suggest that *lin-12(d)* protein is active in the absence of the 'AC-to-VU' signal, is capable of autocrine activation by the signal produced by the isolated cell, or is capable of activation by low levels of the signal present in cellular debris. These results imply not only a functional difference between *lin-12(d)* and *lin-12(+)* proteins, but also a functional similarity among the *lin-12(d)* proteins, because all *lin-12(d)* alleles behave the same way by this laser ablation criterion.

We next analysed the behaviour of *lin-12(d)* mutations in heteroallelic combinations with the *lin-12(n302 n653)* allele (Table 2), which is associated with two point mutations. One, *n302*, is a *lin-12(d)* mutation in the putative extracellular domain; the other, *n653*, is in the putative intracellular domain (see Fig. 1 and Table 2). The phenotype of *lin-12(n302 n653)* homozygotes at 25 °C suggests that *lin-12* activity is strongly reduced: *lin-12(n302 n653)* homozygotes have two ACs (Table 2) as do *lin-12(0)* hermaphrodites. As expected for an allele that reduces *lin-12* activity, *lin-12(n302 n653)* is completely recessive to *lin-12(+)* (Table 2), even in hermaphrodites of *lin-12* genotype *lin-12(n302 n653)/lin-12(n302 n653); mnDp37[lin-12(+)]* (data not shown). Table 2, however, demonstrates that at 25 °C *lin-12(n302 n653)* does not behave as a simple reduced-activity allele in heteroallelic combinations, that is, it does not have *lin-12* activity intermediate between that of *lin-12(+)* and *lin-12(0)*. Instead, in combination with *lin-12(d)* alleles, *lin-12(n302 n653)* seems to have greater activity than a *lin-12(+)* allele. This result suggests that a *lin-12(d)* protein can transactivate or stabilize the protein encoded by *lin-12(n302 n653)*. In addition, the degree of proposed transactivation seems to be *lin-12(d)* allele-specific: although *lin-12(n952)* seems to elevate *lin-12* activity more than *lin-12(n302)* in gene-dosage studies with *lin-12(+)* and *lin-12(0)* alleles, fewer *lin-12(n952)/lin-12(n302 n653)* hermaphrodites than *lin-12(n302)/lin-12(n302 n653)* hermaphrodites display the gain-of-function phenotype (Table 2). This apparent allele-specificity supports the interpretation of a direct association between proteins encoded by *lin-12(d)* and *lin-12(n302 n653)* and, by extension, between those encoded by *lin-12(d)*.

We can perhaps also explain how *lin-12(+)* alleles augment *lin-12(d)* activity, by proposing that a *lin-12(d)* protein can transactivate a *lin-12(+)* protein. But it remains possible that the *lin-12(+)* allele contributes a basal level of *lin-12* activity that can augment *lin-12(d)* activity.

Finally, we postulate that the *lin-12(+)* protein also self-associates to form dimers or multimers, leading to activation. Genetic evidence for self-association of the *Drosophila Notch* protein[11,12], which is similar to the *lin-12* protein in overall predicted structure[6,13,14], has recently been supported by direct biochemical evidence of dimerization[15]. In this context, it is noteworthy that the region of the *lin-12* protein affected in *lin-12(d)* mutants includes a pair of cysteines also present at a corresponding position in Notch, although the extracellular cysteine-poor regions of these two proteins do not display any other notable amino-acid similarity[6]. The two cysteines are also present in a corresponding position in the predicted protein products of other members of the *lin-12/Notch* family, including another *C. elegans* gene, *glp-1* (ref. 16), and genes from *Xenopus* (C. Coffman, C. Kintner & W. Harris, personal communication) and humans (L. Ellisen & J. Sklar, personal communication). These two cysteines—the only two in that region of the protein—may be responsible for the sulphydryl-mediated dimerization of Notch[15].

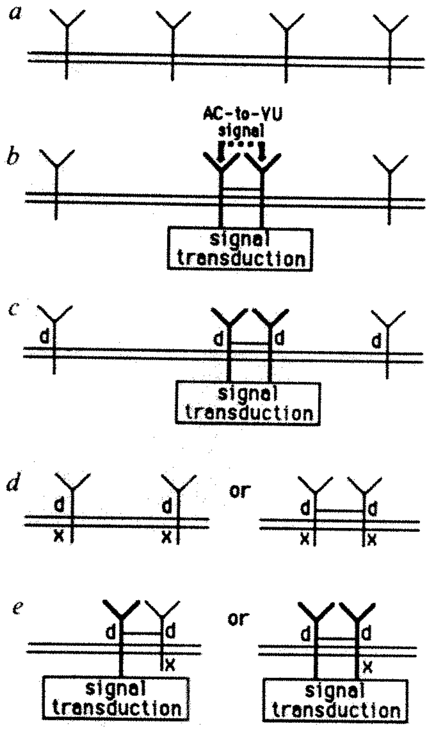

FIG. 2 A model for how dimerization leads to activation of the *lin-12* protein. *a*, In wild type, lin-12 is a receptor that is inactive in the absence of its ligand. *b*, In wild type, the ligand ('AC–VU' signal) promotes dimerization, leading to signal transduction by means of the intracellular domain. Ligand-binding to receptor monomer may cause a conformational change that results in or stabilizes dimerization; alternatively, the ligand may have a more direct role in a cross-linking mechanism. *c*, In a *lin-12(d)* homozygote, dimerization and signal transduction are ligand-independent. The *lin-12(d)* mutations might result in ligand-independent activation of the *lin-12* protein by inactivating a negative regulatory domain or by causing changes in the higher-order structure. *d*, In a *lin-12(n302 n653)* homozygote, the *n653* mutation either prevents homodimer formation or inactivates *lin-12*. *e*, In a *lin-12(d)/lin-12(n302 n653)* heterozygote, heterodimers can form and are active.

TABLE 2 Heteroallelic combinations between *lin-12(n302 n653)* and *lin-12(d)* alleles

lin-12 genotype*	Egl/total†	Percentage of Egl
+/n302 n653	0/203‡	0
n379/n302 n653	143/237	40
n379/+	38/357	11
n379/lin-12(0)	14/252	6
n302/n302 n653	226/296	76
n302/+	101/204	50
n302/lin-12(0)	36/184	20
n952/n302 n653	156/245	64
n952/+	186/299	62
n952/lin-12(0)	63/199	32
n676/n302 n653	202/214§	94
n676/+	183/260	70
n676/lin-12(0)	154/425	36
n137/n302 n653	112/112	100
n137/+	256/289	89
n137/lin-12(0)	88/190	46

All experiments were performed at 25 °C, as the behaviour of *lin-12(n302 n653)* is temperature-dependent (G.S. and I.G., unpublished observations). If the *lin-12(d)* heterozygote and hemizygote data presented here are compared with the data at 20 °C (ref. 4), the penetrance of *lin-12(n952)* and *lin-12(n137)* is lowered and that of *lin-12(n379)* is elevated. The *lin-12(n302)* hermaphrodites are egg-laying defective (Egl), a direct consequence of the transformation of the presumptive AC into a VU[4]. Because the penetrance of the Egl defect is incomplete in *lin-12(d)/lin-12(0)* and *lin-12(d)/lin-12(+)*, wild-type (non-Egl) revertants containing alleles that lower the activity of *lin-12(n302)* may be obtained. The most common intragenic revertant class obtained after ethyl methanesulphonate mutagenesis comprises *lin-12(0)* alleles[4]; *lin-12(n302 n653)* is one of a heterogeneous class of non-null revertant alleles (I.G., unpublished observations). At 25 °C, in the dissecting microscope, *lin-12(n302 n653)* homozygotes all have 2AC (52/52), so it resembles a *lin-12(0)* allele for the AC/VU decision. But *lin-12(n302 n653)* seems to retain some *lin-12* activity: only 52% (71/136) have the characteristic Lin-12(0) ventral protrusion; the remainder have a smaller vulval protrusion. The *lin-12(n302 n653)/lin-12(0)* hermaphrodites resemble *lin-12(n302 n653)* homozygotes.

* We represent here a marker linked to *lin-12* as '*mar*'. Total non-Mar non-Unc self-progeny were counted from heterozygotes of genotype *mar lin-12(d)/unc-36(e251) lin-12(n302 n653); him-5(el467)/+*, *mar lin-12(d)/unc-36(e251); him-5(el467)/+*, or *mar lin-12(d)/unc-36(e251) lin-12(n137 e2034::Tcl); him-5(el467)/+*. For *n379, n302* and *n676, mar* is *dpy-17(e164)*; for *n952, mar* is *unc-32(e189)*; for *n137, mar* is *dpy-19(e1259)*. *lin-12(n137 e2034::Tcl)* is a *lin-12(0)* mutation.

† *Egl*, number of hermaphrodites that display an Egl defect associated with elevated *lin-12* activity (the AC/VU cell fate transformation)[4]. To verify that *lin-12(d)/lin-12(n302 n653)* hermaphrodites displayed this transformation, we examined the progeny of *unc-36(e251) lin-12(n302 n653)/dpy-17(e164) lin-12(n379); him-5(e1467)/+* hermaphrodites in the L3 stage for the presence of an AC: 16/37 (43%) hermaphrodites did not have an AC, and 6/6 of these hermaphrodites were Egl; 21/37 hermaphrodites had one AC, and 7/7 of these hermaphrodites were non-Egl.

‡ 2/203 *unc-36(e251) lin-12(n302 n653)/++* hermaphrodites had an abnormal vulva that affected egg-laying, but the abnormality was not of the sort associated with elevated *lin-12* activity.

§ The *lin-12(n676)* homozygotes, heterozygotes and hemizygotes display the AC to VU transformation but rarely display a 'multivulva' (Muv) phenotype, which is caused by transformation of the vulval precursor cell fates[4]. The 'strong' *lin-12(d)* mutants *lin-12(n950)*, *lin-12(n137)* and *lin-12(n177)* display a Muv phenotype as homozygotes, heterozygotes and hemizygotes; *lin-12(n952)* mutants display a Muv phenotype only as homozygotes. It is therefore striking that most *lin-12(n676)/lin-12(n302 n653)* hermaphrodites displayed a Muv phenotype; the lineages of the vulval precursor cells were not determined.

We incorporate the genetic and molecular findings reported here into our receptor model for *lin-12* function[3] (Fig. 2). According to this model, *lin-12* encodes a receptor that becomes activated on binding of its ligand. The ligand activates the *lin-12* protein by promoting its self-association, and on activation, the intracellular domain induces signal transduction. This model is plausible in view of what is known about biochemically defined receptors. In many cases, the association state of receptors in the membrane controls their activity, and association state is in turn controlled by ligand-binding[17-20]. Although the available data are consistent with the model proposed here, other interpretations are possible. Thus, a future challenge will be to obtain biochemical evidence to confirm or disprove this model. ☐

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RNA-polymerase specificity of transcription of *Arabidopsis* U snRNA genes determined by promoter element spacing

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ALTHOUGH most eukaryotic genes known to be transcribed by RNA polymerase III have intragenic promoter elements, some are similar to genes transcribed by RNA polymerase II in that they have upstream promoters (reviewed in refs 1–4). Transcription of the vertebrate U6 and 7SK RNA genes by RNA polymerase III depends exclusively upon upstream signals, some of which are indistinguishable from the elements essential for polymerase II-specific genes[5-11]. In the plant *Arabidopsis thaliana* the promoter elements for the U6 and U2 small nuclear RNA genes, transcribed by RNA polymerases III and II respectively, are identical, comprising a −30 TATA box and an upstream element specific for small nuclear RNA genes[12,13]. The distance between these elements differs, however. Here we report evidence that this separation is crucial in determining whether the genes are transcribed by polymerase II or III.

Figure 1 illustrates the organization of the upstream promoter elements for the plant U small nuclear (sn) RNA genes transcribed by RNA polymerases II and III (pol II and pol III). In pol II-specific genes the upstream sequence element (USE) and the TATA-like box are centred roughly four DNA helical turns apart, whereas in pol III U6 genes the spacing is about one helical turn less. Hybrid U snRNA genes were prepared to

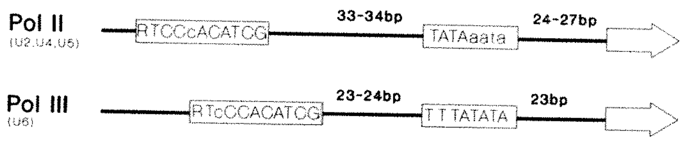
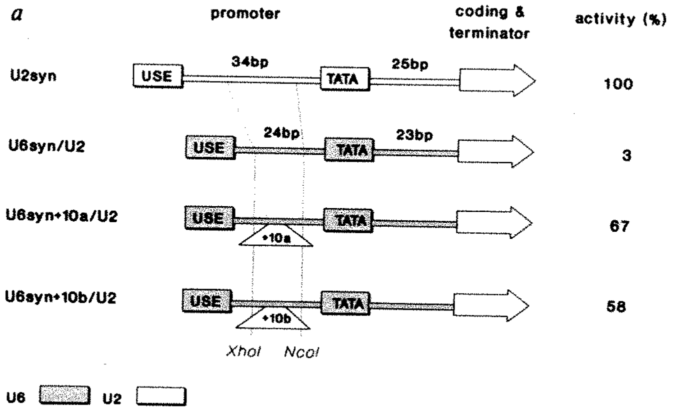


FIG. 1 Conserved upstream elements of pol II- and pol III-transcribed U snRNA genes of *Arabidopsis*. The pol II consensus is based on the sequences of six U2 (ref. 18), one U4 (our unpublished results) and one U5 gene[15]; U1 RNA genes isolated from bean, soybean and tomato contain the same conserved elements at similar positions[21,22]. The pol III consensus is based on the sequences of three active U6 genes (F. W. and W. F., manuscript submitted). Elements found in both pol II and pol III promoters are boxed. Nucleotides not conserved in all genes are in lower case. The coding region is indicated by an arrow. For both U2 and U6 genes of *Arabidopsis* the USE and TATA seem to be the only essential promoter elements and the TATA elements of U2 and U6 genes are exchangeable. The evidence that U2, U4 and U5 genes are pol II-specific is based on the presence of m_3G caps in the RNA products and on the sensitivity of transcription in protoplasts to α-amanitin. Transcription of the U6 genes is resistant to α-amanitin and terminates within stretches of T residues, which function as pol III terminators[12,13].

examine whether a pol III promoter can be converted into a pol II promoter and vice versa, by changing the distance between the elements. In the construct U6syn/U2 (Fig. 2*a*) the promoter of the model U6syn gene was fused with an *Arabidopsis* U2 gene lacking its own promoter[12]. The resulting gene contains a 70-base-pair (bp) U6syn promoter followed by 196 bp of the coding and 264 bp of the downstream noncoding U2 gene sequence. Transfection of the hybrid U6syn/U2 gene into protoplasts of *Nicotiana plumbaginifolia* resulted in accumulation of only trace levels of U2 RNA (Fig. 2*b*). This observation is not unexpected. If RNA synthesis from this construct were indeed initiated by pol III, transcription would probably terminate in the coding region of the U2 gene where stretches of T residues, which act as pol III terminators[14], are present. The accumulation of mature U2 RNA may also be prevented by the inability of pol III to terminate transcription at the U2 gene termination signal[15] or by inappropriate RNA capping[16].

We prepared two derivatives of the U6syn/U2 hybrid, U6syn+10a/U2 and U6syn+10b/U2, each having an additional 10 bp of different sequence inserted between the USE and TATA box (Fig. 2*a*). Transfection of these genes resulted in accumulation of U2 RNA at a level comparable to that seen



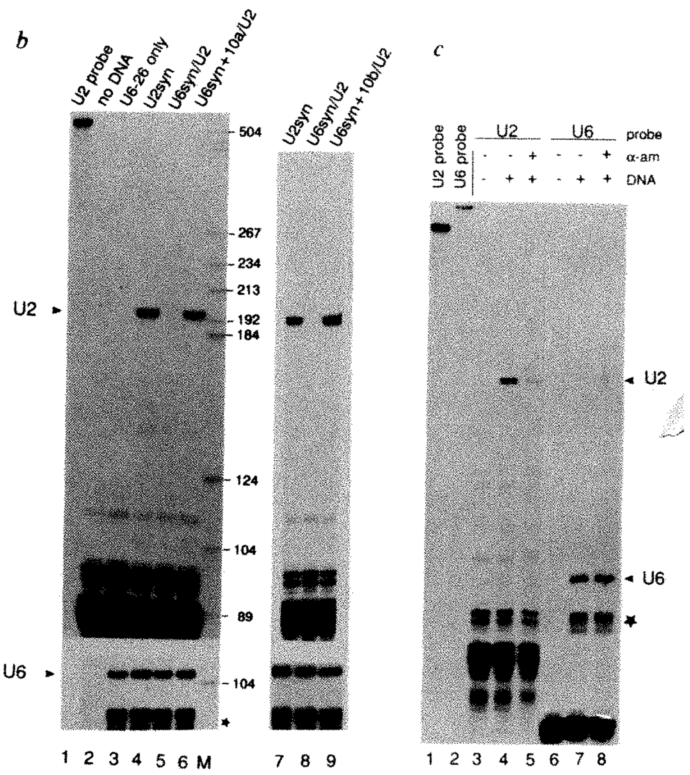


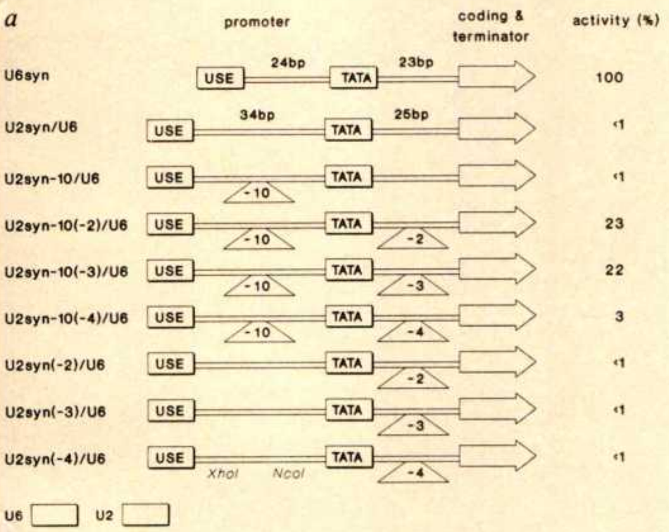
FIG. 2 Conversion of the U6 gene promoter into a pol II-specific promoter. *a*, Schematic representation of the U2syn gene and of the hybrid U6syn/U2 genes. The U2syn and U6syn gene sequences of the hybrid genes are shown as open and stippled boxes, respectively. The relative activities of the different genes are indicated (averages from three independent transfections). *b*, RNase A/T_1 mapping. Protoplasts were transfected with plasmid pU6-26 (containing reference U6 gene) alone (lane 3) or with pU6-26 plus the genes indicated above lanes 4-9. The upper and lower panels show mapping with U2 and U6 probes, respectively. Two different U2-specific probes, the wild-type U2 gene probe (lanes 4-9) and the U6syn/U2-specific probe (data not shown; these probes differ in sequences upstream of position +1) yielded identical protected fragments, indicating that initiation occurred at the A at position +1 and that transcripts were properly terminated. M, size markers (in nucleotides). The 93-97-nucleotide bands (*) result from the partial digestion of the U6 RNA-specific hybrid by RNase A at the AU-rich 3′ end. *c*, Effect of α-amanitin on expression of the gene U6syn+10a/U2. Protoplasts were cotransfected with pU6syn+10a/U2 and pU6syn and incubated in the presence or absence of 50 μg ml^{-1} α-amanitin. Mapping with U2 and U6 probes is in lanes 3-5 and 6-8, respectively.

METHODS. The gene U2syn is a derivative of U2.SP, the *Arabidopsis* U2 RNA gene that contains a synthetic 98-bp promoter; pU2syn was constructed similarly to pU2.SP (ref. 12). The U2syn gene differs from U2.SP by four nucleotide substitutions in the −22 to −8 region that create *NdeI* and *AatII* sites (Fig. 3*b*). The activities of the U2.SP, U2syn and the authentic U2 gene are comparable[12]. The plasmid pU6syn/U2 was constructed similarly to pU2syn (ref. 12). The U6syn promoter sequence (position −70 to −1; see Fig. 3) was assembled from five oligonucleotides and inserted into pU2.2/ClaI (ref. 12) pre-cut with *Bam*HI and *Cla*I. Mutated constructs U6syn+10a/U2 and U6syn+10b/U2 were generated from U6syn/U2 by replacement of the *XhoI*-*NcoI* fragment with a longer linker having the sequence CTCGAGTAAAAATAAGAAAGATTCCATGG or CTCGAGTTATGGAATTTCTTCTACCATGG. Expression was tested in protoplasts of *N. plumbaginifolia*[26,27]. Subsaturating amounts (10 μg) of plasmids were used. RNA was prepared 24 h after transfection and used (3 μg) for RNase A/T_1 mapping[26,27]. The U6 and U2 gene-specific antisense RNA probes, of 440 and 510 nucleotides, were synthesized with SP6 polymerase using [$\alpha-{}^{32}$P]GTP and pU6-26 (linearized with *Bst*XI) or pGEM2.U2.2 (linearized with *Hin*dIII)[18] as templates. Protected fragments were quantitated[26,27] and activity normalized for transfection efficiency as measured by expression of the reference gene.

with the gene U2syn, in which synthesis of U2 RNA is driven by a synthetic U2 gene promoter (Fig. 2*b*). Transcription of the gene U6syn + 10a/U2 was inhibited by α-amanitin as expected[17] for a gene transcribed by pol II; transcription of the cotransfected control U6 gene was α-amanitin resistant (Fig. 2*c*). It is possible, therefore, to change the pol III-specific U6 gene promoter into an efficient pol II-specific promoter by inserting 10 bp of DNA between the USE and the TATA-like box. It should be noted that each of the inserted sequences differs substantially from the sequences present in this region in the authentic U2 genes[18].

To study the pol II to pol III promoter conversion we have constructed a hybrid gene, U2syn/U6, in which the 98-bp synthetic promoter[12] of the gene U2syn is fused with the coding and terminator regions of the gene U6syn. We also made a U2syn/U6 derivative, U2syn − 10/U6, in which the distance between the USE and TATAAATA was shortened by 10 bp to conform with the spacing in pol III-specific promoters (Fig. 3*a*). Neither of the two genes was active in synthesis of U6 RNA (Fig. 3*c*). We have noted that the distance between the TATA-like sequence and the transcription start site is 1-4 bp shorter in U6 genes as compared with the pol II-specific genes (Fig. 1) and, in particular, 2 bp shorter than in the genes U2syn/U6 or U2syn − 10/U6 (Fig. 3*b*). We have therefore prepared three variants of each of these two genes, in which the TATA to +1 distance is shortened by 2, 3 or 4 bp (Fig. 3*a* and *b*). None of the constructs of the U2syn/U6 series (U2syn (−2, −3 or −4)/U6) was active but two of the constructs containing, in addition, a 10-bp deletion between the USE and TATA, U2syn − 10(−2)/U6 and U2syn − 10(−3)/U6, yielded U6 RNA (Fig. 3 *c*). The U6 RNA synthesis from U2syn − 10(−2)/U6 (Fig. 3*d*) and U2syn − 10(−3)/U6 (data not shown) was α-amanitin-resistant as expected for genes transcribed by pol III. Hence, it is also possible to change a pol II promoter into a pol III promoter by manipulating the spacing between the USE and TATA elements, provided that the distance between the TATA-like sequence and the initiation site conforms to the U6 gene consensus.

It is unlikely that the 1-4-bp difference in the TATA to +1 distance found between pol II and pol III U snRNA genes is important for polymerase selection. The gene U6syn + 10/U2, for example, has a pol III-like TATA to +1 spacing, but is very efficiently transcribed by pol II (Fig. 2). It is more probable that pol II and pol III themselves differ slightly in the properties responsible for specifying the TATA to +1 distance. It has been demonstrated that factors other than the TATA box-binding



b

GENE	TATA/+1 DIST. (bp)	DNA SEQUENCE -10 -1 +1	
U6syn	23	TATA ATGATC--ATGTCGACATATCGATT	GTCCCTTCGG
U2syn/U6 and U2syn-10/U6	25	TATA ACTCATATGCTGGTGACGTCAATTC NdeI AatII	GTCCCTTCGG
U2syn(-2)/U6 and U2syn-10(-2)/U6	23	TATA ACTCAT**GCTGGTGACGTCAATTC	GTCCCTTCGG
U2syn(-3)/U6 and U2syn-10(-3)/U6	22	TATA ACTCA***GCTGGTGACGTCAATTC	GTCCCTTCGG
U2syn(-4)/U6 and U2syn-10(-4)/U6	21	TATA ACTCA****CTGGTGACGTCAATTC	GTCCCTTCGG

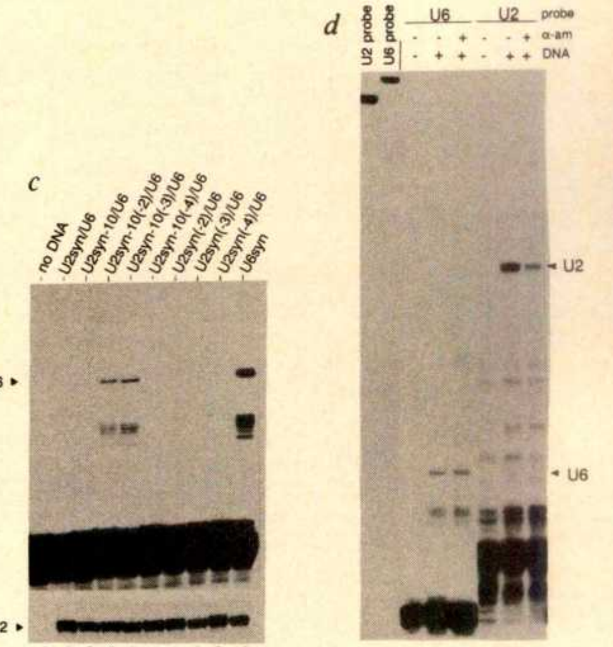


FIG. 3 Conversion of the U2 gene promoter into a pol III-specific promoter. *a*, Schematic representation of different genes and their activities (for more details see text and Fig. 2*a*). *b*, sequences of the −25 to +10 regions of the genes in (*a*). *, Nucleotides deleted in some mutated constructs. The *Nde*I and *Aat*II sites in U2syn/U6 are underlined. The distance between the TATA element (boxed) and position +1 is indicated. *c*, RNase A/T_1 mapping with U6 and U2 probes. Plasmids used for contransfection with pU2.2, which contains the reference U2 gene[12], are indicated at the top. Mapping of U6 RNAs transcribed from these plasmids was carried out with probe U6-26 (lanes 2-10), which is complementary to the upstream nucleotides in genes U6-26 and U6syn but not in genes of the U2syn/U6 series and with the probe specific for the gene U2syn/U6 (data not shown). The protected fragments were of identical lengths, indicating that initiation occurred at the G at position +1 and that transcripts were properly terminated. *d*, Effect of α-amanitin. Protoplasts were contransfected with pU2syn − 10(−2)/U6 and pU6syn. Mapping with U6 and U2 probes, lanes 3-5 and 6-8 respectively.

METHODS. The 188-bp U6syn sequence, assembled from 10 overlapping oligonucleotides, is described in detail elsewhere[13]. The 70-bp upstream region of U6syn contains the USE and TTTATATA elements at the conserved positions. The remaining upstream sequence is mostly unrelated to that of the authentic U6 genes and contains several restriction sites. The coding region is identical to the wild-type sequence and a stretch of T residues is included downstream as a terminator. The U6syn was transcribed with 60-65% efficiency compared with the authentic U6 RNA genes. The parent U2syn/U6 hybrid gene, including 98 bp of U2syn promoter (see Fig. 2) fused with the coding and terminator regions of U6syn, was prepared by annealing and ligation[26] of 11 oligonucleotides. Synthetic U6syn and U2syn/U6 fragments were inserted into plasmid pTZ19R pre-cut with *Bam*HI and *Sph*I. The variants U2syn (−2, −3 or −4)/U6 were obtained by cleavage of pU2syn/U6 with *Nde*I, followed by limited digestion with mung bean nuclease and religation. The variants having a 10-bp deletion in the USE/TATA region were obtained by replacement of the 29-bp *Xho*I-*Nco*I fragment[12] with the shorter linker CTCGAGTAAAAATCCATGG.

protein (possibly RNA polymerase itself) 'measure' this distance during transcription by pol II[19,20]. The inability of pol III to initiate transcription from the U2syn−10/U6 and U2syn−10(−4)/U6 genes may be due to the absence of a pyrimidine-purine dinucleotide located at the correct (22–23 bp) distance from the TATA box (Fig. 3*b*). Transcription of all plant U snRNA genes starts at a purine residue preceded by a pyrimidine[15,18,21,22]. Transcription of the U6 gene of *Xenopus* has similar requirements[6].

Our experiments do not formally eliminate the possibility that intragenic sequences also contribute to RNA polymerase selection. But the identification of two other pol III-specific gene classes in *Arabidopsis* having USE/TATA spacing similar to that of U6 genes (T. Kiss, Ch. Marshallsay and W. F., manuscript in preparation) and the demonstration that the intragenic A box-like sequence found in *Arabidopsis* U6 genes is not required for transcription[13] make this possibility unlikely.

The mechanism by which differences in spacing between two promoter elements in plant U snRNA genes determine the choice of RNA polymerase is not known. The mode of interaction between the proteins that bind the USE and TATA may depend on the spacing between them, giving rise to two alternative surfaces recognized by the appropriate polymerase complex or by an additional protein acting in between. Alternatively, distinct TATA or USE factors may interact with pol II and pol III promoters, specificity being governed by the proximity of the protein bound at the neighbouring site.

The structure of U snRNA gene promoters and the factors that define polymerase specificity are different in plant and vertebrate cells. Class II and class III promoters in vertebrates contain two common upstream elements, DSE and PSE, which are sufficient for transcription of the U2 gene by pol II (reviewed in refs 23, 24). The U6 genes contain an additional element, an AT-rich TATA-like box that confers pol III specificity[6,9]. In plants, both classes of U snRNA genes contain essential −30 TATA-like elements and it will be of interest to determine whether the same or different TATA factors participate in pol II- and pol III-specific transcription.

Our results support the idea that polymerases II and III evolved from a common ancestor[25], as did pol II and pol III U snRNA gene promoters[6], and that one mechanism of promoter diversification in eukaryotes involved changes in the spacing between promoter regulatory elements. ☐

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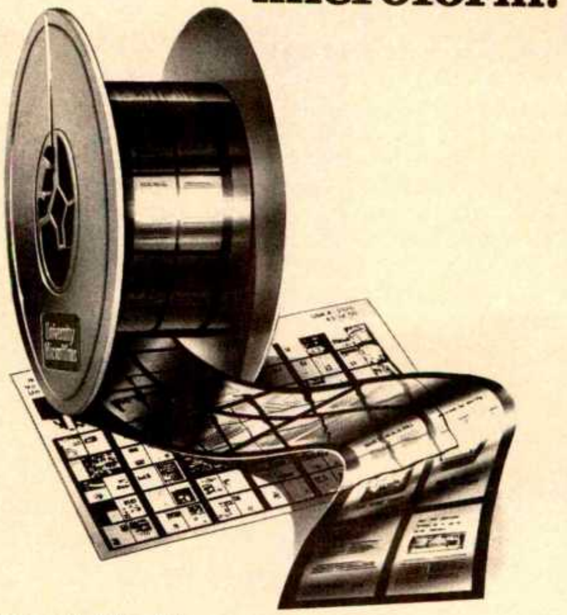

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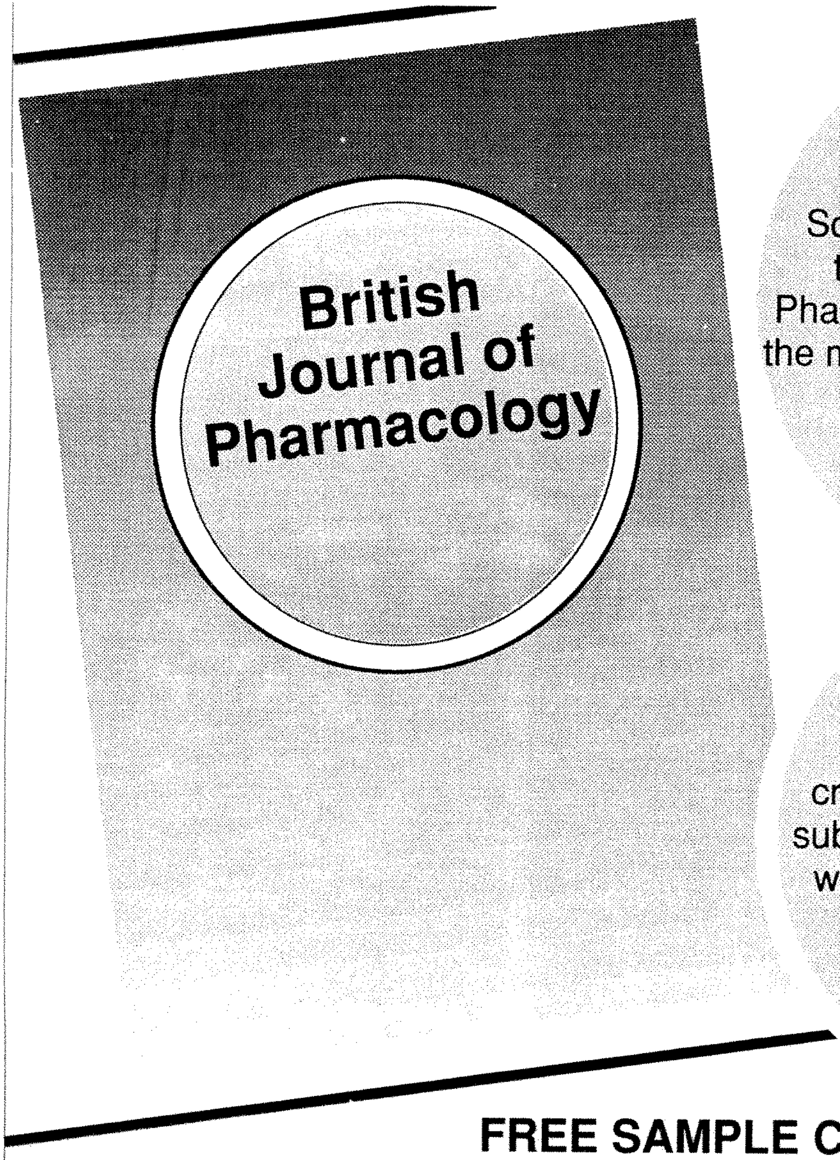

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REF: DG/12/90

Organization: ICARDA, one of the 13 international centers supported by the Consultative Group on International Agricultural Research, cooperates with national programs in West Asia and North Africa (WANA) to develop sustainable improvements in dryland cereals, food legumes, farm resources management, pasture, forage, and livestock production. ICARDA's multi-disciplinary staff of over 60 senior scientists and 600 technical and support personnel has an international mandate for barley, faba bean, and lentil production improvement and — with other centers — a joint regional mandate for wheat and chickpea production improvement.

Position: The Water Management/Supplemental Irrigation Specialist is a member of a multi-disciplinary team conducting research in agricultural systems of supplemental irrigation and water harvesting. Responsibilities include:

- Collaborate with national and international scientists to plan, design, implement, manage, and evaluate supplemental irrigation and water harvesting projects aimed at technology transfer in WANA;
- Participate in multi-disciplinary supplemental irrigation and water harvesting research for cereals, pulses, forages, and pasture management;
- Prepare a variety of documents and reports;
- Work with agricultural economists, agronomists, and other team members;
- Assist with organizing and conducting training events.

Qualifications: Earned PhD in agricultural engineering, civil engineering, agronomy or subject involving irrigation; minimum of 2 years relevant research experience; strong background with advanced knowledge and experience in supplemental irrigation and water harvesting; ability to work collaboratively with scientists of different disciplines and nationalities; teaching experience and familiarity with microcomputers and mapping; fluent in spoken and written English; under 60 years of age.

Post: The position reports to the leader, Farm Resources Management Program, and is based at ICARDA's modern headquarters and main research station located in a rural setting 35 km from Aleppo, Syria.

N_2-FIXATION SPECIALIST/SOIL MICROBIOLOGIST

REF: DG/09/90

Organization: ICARDA, one of 13 international centers supported by the Consultative Group on International Agricultural Research, cooperates with national programs in West Asia and North Africa to develop sustainable improvements in dryland cereals, food legumes, farm resources management, pasture, forage and livestock production. ICARDA's multi-disciplinary staff of over 60 senior scientists and 600 technical and support personnel has an international mandate for barley, faba bean, and lentil production improvement and, with other centers, a joint regional mandate for wheat and chickpea production improvement.

Position: The N_2-Fixation Specialist/Soil Microbiologist is part of a multi-disciplinary team seeking to improve sustainability of regional dryland farming systems with specific application to legume biological nitrogen fixation and nitrogen cycling. Main responsibilities include:

- Develop and implement a research program involving role and maximization of fixed nitrogen in crop rotation involving forage and food legumes;
- Strengthen relationships with national programs and assist in developing research capability through training and cooperative projects;
- Produce technical reports and training material;
- Establish effective collaboration with other centers of excellence to augment regional efforts.

Qualifications: Earned PhD in agronomy, soil science, or soil microbiology; at least 5 years research experience in legume N_2 fixation, N cycling, and 15N technique; practical experience in developing countries and/or dryland farming systems desirable. Fluency in written and spoken English is essential.

Post: The position reports to the leader, Food Legume Improvement Program, and is based at ICARDA's modern headquarters and main research station located in a rural setting 35 km from Aleppo, Syria. Arabic and/or French capability would be advantageous.

LEGUME PATHOLOGIST

REF: DG/10/90

Organization: ICARDA, one of the 13 international centers supported by the Consultative Group on International Agricultural Research, cooperates with national programs in West Asia and North Africa to develop sustainable improvements in dryland cereals, food legumes, farm resources management, pasture, forage, and livestock production. ICARDA's multi-disciplinary staff of over 60 senior scientists and 600 technical and support personnel has an international mandate for barley, faba bean, and lentil production improvement and, with other centers, a joint regional mandate for wheat and chickpea production improvement.

Position: The Legume Pathologist works within a multi-disciplinary team seeking ways to stabilize and increase productivity of cool season food and forage legumes through improved host resistance and alternative controls for common diseases, with major emphasis on basic and applied research related to Ascochyta blight, root-rot, rust, and wilt in lentil and chickpea. Main responsibilities include:

- Develop and implement collaborative research programs with breeders on host-plant resistance and appropriate alternative control measures;
- Produce technical reports and training material;
- Strengthen relationships with national programs and assist in developing research capability through training and cooperative projects;
- Establish effective collaboration with other centers of excellence for research on mechanisms of resistance and application of biotechnology to plant disease management.

Qualifications: Earned PhD in plant pathology with at least 5 years research experience in host-plant resistance and alternative control measures. Fluency in written and spoken English is essential. Experience with cool season legumes in developing countries is desirable.

Post: The position reports to the leader, Food Legume Improvement Program, and is based at ICARDA's modern headquarters and main research station located in a rural setting 35 km from Aleppo, Syria. Arabic and/or French capability would be advantageous.

Benefits: Salary paid in US dollars based on experience and qualifications. Benefits include housing allowance, paid home leave travel, a retirement and health package, provided auto, free enrollment for dependents at the ICARDA-administered international school (K-12), and other internationally competitive conditions of service. **Application:** Qualified applicants are invited to send: 1) a curriculum vitae with recent salary history; 2) names, addresses, and telex numbers for three professional referees; 3) photocopies (non-returnable) of other relevant supporting documents, to Dr Aart van Schoonhoven, Deputy Director General/Research ICARDA, PO Box 5466, Aleppo, Syria. Telex: 331208 ICARDA SY, or 331263 ICARDA SY, or 331206 ICARDA SY. Telephone: (963-21) 213433, 213477, or 234890. **Reference:** Please quote position nos on applications. **Application deadline:** Positions open until qualified candidates apply. **Announcement date:** 6 May 1990.

(W7529)A

ICARDA is an Equal Opportunity Employer

WORKSAFE AUSTRALIA
(NATIONAL OCCUPATIONAL HEALTH AND SAFETY COMMISSION)

Australia is introducing a new scheme of chemical notification and assessment to help ensure that hazardous substances are appropriately handled and regulated.

The National Occupational Health and Safety Commission (Worksafe Australia) is a tripartite body which leads national efforts to provide healthy, safe and productive working environments and to reduce the incidence and severity of occupational injury and disease.

The Commission is seeking an experienced executive to direct the Chemicals Notification and Assessment Scheme and administer the day-to-day operation of the Industrial Chemicals (Notification and Assessment) Act 1989. This act establishes a national system of notification and assessment of the potential hazards of industrial chemicals to workers, the community and the general environment.

DIRECTOR
Chemicals Notification and Assessment Scheme
(Senior Executive Band 2)
Sydney

Duties: Under the direction of the Chief Executive Officer, manage the administrative operations and exercise the legislative responsibilities of the Act. Maintain an inventory of chemical substances used in Australia and develop and manage the branch responsible for this work.

Qualifications: Applicants should have highly developed managerial and leadership ability, together with demonstrable knowledge, experience and mature judgement about the use and effects of toxic substances.

The remuneration package for this position totals approximately $A100,000 of which $A69,206 is the salary component.

Australian Public Service conditions of service apply and include four weeks annual leave with bonus payment, cumulative sick leave benefits, long service leave after ten years continuous service and a contributory superannuation scheme. In some circumstances, fares and removal expenses to take up duty and a short term accommodation allowance are payable.

The successful candidate will be offered appointment on a fixed term basis not exceeding five years.

Further information on this position is available from the Chief Executive, Dr Edward Emmett, (02) 265 7500. Duty statement, job description and selection criteria may be obtained from Ms Abi Gough on (02) 265 7454.

Applications addressing the selection criteria and giving full details of experience and qualifications should be forwarded to the:

Personnel Officer (Recruitment)
WORKSAFE AUSTRALIA
GPO Box 58, Sydney, NSW 2001, AUSTRALIA

Applications close: Two weeks from the date of this advertisement.

Worksafe Australia has a policy of a smoke-free working environment, and is an Equal Opportunity Employer (W7550)A

UNIVERSITY OF DUNDEE
DEPARTMENT OF ANATOMY AND PHYSIOLOGY
TEMPORARY LECTURESHIP IN ANATOMY

Applications are invited from experienced medical, dental, or suitably qualified science graduates for a Temporary Lectureship in Anatomy. The appointment will be for one year from 1 October 1990 but an earlier starting date can be arranged.

Salary will be on the Lecturer Grade A scale (£12,086 - £16,755).

Further particulars from, and applications in writing with CV (6 copies or, if posted overseas, one copy in a suitable format for photocopying) and the names and addresses of three referees to, the **Personnel Office, The University, Dundee, DD1 4HN. Please quote reference EST/32/90/N.**

Closing date: 3 August 1990. (4141)A

UNIVERSITY OF GLASGO
Wellcome Laboratories for Experimental Parasitology
POSTDOCTORAL RESEARCH ASSISTANT

Applications are invited for the above post to work on the cloning of gen
encoding secreted antigens and proteinases of nematode parasites. The proje
is funded by the Wellcome Trust for three years beginning October 1990. T
successful applicant will join a laboratory working on a variety of projects on
immunochemistry and biochemistry of parasitic nematodes. Experience
molecular biology, biochemistry, or related fields would be advantageou
particularly if in gene cloning.

The post is available from October 1990, but a later date could be negotiated
necessary. Starting salary will be on the University RA 1A scale (stimulated by
increments) from £12,792 to £13,495, depending on age and experience, an
appropriate annual increments thereafter.

Applications including curriculum vitae and the names, addresses and tel
phone numbers of at least two adademic referees should be sent as soon a
possible to **Dr Malcolm Kennedy, Wellcome Laboratories for Experiment
Parasitology, University of Glasgow, Bearsden, Glasgow G61 1HQ** to who
informal enquiries can be made on 041-339 8855 Extn 5819 or 6968. (4138)A

The Queen's University of Belfast
RESEARCH FELLOW
Department of Agricultural Botany

The above post, located at the Plant Testing Station, Crossnacreevy
Belfast, is available initially for one year from 1 September 1990 fo
work on cereal variety evaluation based on field experiments con
ducted at various sites throughout Northern Ireland. Data on yield
quality and agronomic characteristics are collected, processed an
interpreted to provide information on varieties suitable for produc
tion in Northern Ireland. Duties will include management of the fiel
trials, data collection and processing and the formulation of report
on varieties. The appointee will be involved in communicatin
information to various sectors of the agricultural industry in Norther
Ireland.

Applicants must hold a Degree in Agricultural Botany or a relate
subject and a PhD or have five years postgraduate research experience. Experience of staff management and computing is essential.

Salary scale: £10,458-£12,381 per annum (under review), placing depending on age, qualifications and experience.

Applicants, quoting ref. 90/HH, should submit a curriculum vitae, including the names and addresses of two referees to the **Personnel Officer, The Queen's University of Belfast, Northern Ireland, BT7 1NN.** Closing date: 27 July 1990.

The University is an Equal Opportunity employer. The University may decide to interview only those applicants who appear, from the information available, to be the most suitable, in terms of experience, qualifications and other requirements of the post. (4128)A

THE CHARING CROSS SUNLEY RESEARCH CENTRE
IMMUNOLOGY UNIT
POSTDOCTORAL FELLOWS

Two Vacancies:

1) **Molecular Biologist** to work in a group studying the structure and function of the receptors for tumour necrosis factor and their role in disease processes. Experience in cDNA cloning, mammalian cell expression and PCR an advantage.

2) **Cell Biologist** to work in a group studying cytokine production and regulation of endothelium by cytokines. Experience in endothelial cell culture and/or coagulation is desirable.

For further details contact Prof. M. Feldmann (project 1 or 2), Dr. C. Haworth (2) or Dr. F. Brennan (1).

Salaries in the range £12,225-£18,432 (Research Scale 1A) depending on age and experience.

Closing date: (4 weeks after publication date).

Applications, including a full C.V. and the names and addresses of two referees should be sent to:

Mrs. J. Brewer
The Charing Cross Sunley Research Centre
1 Lurgan Avenue, Hammersmith
London W6 8LW
(Tel: 081-741 8966)

(4129)A

Cancer Research Campaign

- We are the biggest funder of cancer research scientists, supporting some 700 projects in universities, medical schools and research institutes throughout Britain
- We have developed a strong interface between research and clinical therapy, facilitating the effective development and implementation of cancer treatments
- Of the nearly £40 million we raised through voluntary contributions in1989, 92% went directly into research - accounting for around 40% of all cancer research in the UK.

INSTITUTE OF CANCER RESEARCH, (University of London)

POST-DOCTORAL SCIENTIST, Sutton, Surrey

Applications are invited for a postdoctoral research fellow to join a multidisciplinary team working within the new Molecular Carcinogenesis Section. Major interests of the team include (i) the isolation of new oncogenes that are involved in the development of human soft tissue tumours and (ii) the characterisation of a novel growth receptor encoded by the MET protooncogene and the identification of the growth factor that binds to this receptor. The person appointed will be expected to take scientific responsibility for work on the MET growth factor receptor but depending upon background and interests will have the opportunity to become involved in other research projects. Prior experience in molecular biological techniques, although by no means essential, would be an advantage. The post is for three years in the first instance. Salary will be in the range £12,108 to £17,022 inclusive, according to experience.

Applications should be sent in duplicate, with the names and addresses of two referees to the Personnel Officer, Institute of Cancer Research, 17A Onslow Gardens, London SW7 3AL, quoting reference number 7.90.S.N.17.

THE BEATSON INSTITUTE FOR CANCER RESEARCH

RESEARCH SCIENTIST, Glasgow

There is a vacancy for a scientist in a group studying the mechanisms regulating changes in gene expression and leukaemogenesis in human myeloid cells. Candidates should have experience of cell and molecular biology techniques, an interest in the regulation of gene expression in eukaryotes, and a willingness to explore new directions. The appointment will be within a salary range of £12,086 to £22,311 per annum at a point appropriate to age and relevant experience, and will be for three to five years, with possibility of extension depending on the incumbent's progress and proven ability.

Applications, with CV and names and addresses of three referees, should be sent to Dr G D Birnie, The Beatson Institute for Cancer Research, Garscube Estate, Switchback Road, Bearsden, Glasgow G61 1BD, from whom further details can be obtained.

CRC HUMAN CANCER GENETICS RESEARCH GROUP,

University of Cambridge

We are a major CRC-funded group working at the interface between the clinical and molecular genetics of cancers in man. We are based in new laboratories in the University Department of Pathology in the centre of Cambridge.

RESEARCH TECHNICIAN

The successful applicant will work closely with a team leader on a project to map genes involved in the development of human cancers, in addition to a share of laboratory duties in support of the team as a whole.

Experience and proven ability to get results with basic molecular genetic techniques are required. This post is suitable for someone who enjoys getting techniques to work; and further training will be given. It is not suitable for someone wishing to do a Ph.D. while in post.

Appointment for 3 years in the first instance. Salary according to age and experience in range £9,672 to £11,898 (under review).

RESEARCH ASSISTANT

The successful applicant will be responsible for developing the molecular genetic aspects of our studies of human ovarian and breast cancer, including the development of new highly polymorphic probes based on PCR techniques. Training and supervision will be available if required.

Applicants may or may not have a Ph.D. Experience and demonstrated success with basic molecular genetic techniques (blotting, genomic cloning) is essential.

Appointment for 3 years in the first instance. Salary according to age and experience in range £11,399 to £16,755.

Applications for both the above positions should be made to Dr Bruce Ponder, CRC Human Cancer Genetics Research Group, Department of Pathology, University of Cambridge, Tennis Court Road, Cambridge CB2 1QP.

Applicants are advised that smoking is prohibited in the majority of our premises.

Fighting cancer on all fronts. (4154)A

KING'S COLLEGE SCHOOL OF MEDICINE AND DENTISTRY of King's College London

Department of Immunology

RESEARCH ASSISTANT IN DIABETIC RESEARCH

Applications are invited for a 2 year post to undertake a study of the immunopathogenesis of diabetes in the non-obese diabetic mouse. The post is based in the Department of Immunology where the successful candidate will join a group dedicated to elucidating the role of T lymphocytes and their soluble products in the immunopathogenesis of insulin dependent diabetes. The post would suit a young graduate with an enquiring mind for whom a career in research is the prime motivation. King's has excellent post-graduate facilities and the salary will be according to the Research Assistant's scale. For further details and informal discussions please contact Dr D Vergani or Dr M Peakman (telephone 071 326 3588). Application in the form of a CV naming two referees should be sent to the **Secretary of the Medical School, Bessemer Road, London SE5 9PJ**, by 28 July 1990. (4151)A

POSTDOCTORAL POSITION

Jointly administered by Department of Environmental Engineering, California Institute of Technology and Department of Experimental Cardiology, Huntington Medical Research Institutes, Pasadena, CA. Purpose to identify nitric oxide or its various precursor materials in biological systems (from endothelial cells). The findings will be correlated with the biopharmacological effect of these nitrogenous substances using bioassays systems. Experience in chemistry and interest in biomedical problems desirable. Starting date Summer or early Fall. Send applications to **Richard J. Bing, M.D., Huntington Medical Research Institutes, 734 Fairmount Avenue, Pasadena, CA 91105.** (818) 397-5451. *EOE/AA.* (NW5524)A

THE INTERNATIONAL ATOMIC ENERGY AGENCY

SEEKS

Head, of Soil Fertility, Irrigation and Crop Production Section for the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, Vienna, Austria.

Applicants should hold a Ph.D. degree in soil science, plant nutrition, root biology, soil microbiology or a related subject and have 15 years of research experience including work with isotopes, radiation and biotechnological techniques in one or more of the above topics. Relevant experience in developing countries would be an asset. Experience with international programmes and adminstration desirable.

The Head of the Soil Fertility, Irrigation and Crop Production assists in formulating and guiding the Joint FAO/IAEA programme to assist Member States to apply nuclear and related techniques in research relevant to maximising efficiency in the use of soil resources and applied fertilizer and water using agronomic and plant genotypic approaches, to enhancing soil fertility via management of biological nitogen fixation of grain legume and trees, and to soil conservation.

Tax free emoluments of approximately $US65,600 p.a. Initial contract for three years. Additional allowances for dependants. Six weeks annual leave. Travel and removal expenses paid. Applicants should send their curriculum vitae quoting Vacancy Notice No. 90/028 to the **Division of Personnel, International Atomic Energy Agency, P.O. Box 100, Wagramerstrasse 5, A-1400 Vienna, Austria, before August 24th 1990.** (W7542)A

University of Sheffield

DEPARTMENT OF MEDICINE & PHARMACOLOGY

TWO NON-CLINICAL LECTURESHIPS IN MOLECULAR MEDICINE

Applications are invited from candidates with good records of academic achievement for two Non-Clinical Lectureships:

For one post, candidates must have a sound understanding and experience of advanced molecular biology techniques and DNA analysis, particularly in relation to the use of the polymerase chain reaction DNA amplification procedure. Ref. MAP 302A/G.

Candidates for the other post should have a background in one of the following: DNA cloning and sequencing; gene regulation studies; protein biochemistry; cytokine/growth factor cell biology; molecular virology; biochemical immunology. Applicants must be capable of developing and leading a competitive research group within one of the above areas. **Ref. 302B/G.**

Salaries on Grade A (£10,458-£15,372 pa, under review) or Grade B (£16,014-£20,469 pa, under review).

Further details from Director of Personnel Services, The University, Sheffield, S10 2TN, to whom applications including a full CV and the names/addresses of three referees (six copies of all documents), should be returned by **15 August 1990.** Please quote appropriate reference.

An Equal Opportunity Employer

(4142)A

at the leading edge

CHAIR, DEPARTMENT OF ENTOMOLOGY & APPLIED ECOLOGY

Applications are invited for Professor and Chair, Department of Entomology and Applied Ecology, University of Delaware. We seek candidates who will continue superior achievement in research and who demonstrate commitment to quality undergraduate and graduate instruction. Research speciality is open. Persons using insect models in disciplines other than entomology are encouraged to apply.

Salary and fringe benefits are competitive. Committed to academic excellence, the University of Delaware is a mid-sized comprehensive land grant institution with convenient access to major museum, USDA and industrial research facilities. The position is available July 1, 1991; final application deadline is September 15, 1990. The search will continue until the position is filled.

Send full curriculum vitae, including complete funding and publication record, and names, addresses, and telephone numbers of four references to: **Dr. John Dohns, Search Committee Chair, Entomology and Applied Ecology, University of Delaware, Newark, DE 19717-1303.**

The UNIVERSITY OF DELAWARE is an Equal Opportunity Employer which encourages applications from Minority Group Members and Women.

(NW5514)A

UNIVERSITY OF WARWICK

Nonlinear Systems Laboratory

EXPERIMENTAL RESEARCH FELLOW TO STUDY CHAOS

A SERC-sponsored postdoctoral position to conduct experimental investigations with Dr C P King of chaotic advection in a simple fluid.

Experimental experience is required. Experience in fluid mechanisms or chaotic dynamical systems desirable but not essential.

The appointment is for two years and is available from 1 October 1990. Salary will be up to £13527 on the Research Range IA scale: £10458 – £16665 pa (under review) depending on age and experience.

Send curriculum vitae, description of interests and names and addresses of two referees to **Dr G P King, Nonlinear Systems Laboratory, Mathematics Institute, University of Warwick, Coventry CV4 7AL. Closing date 27 July 1990.**

AN EQUAL OPPORTUNITIES EMPLOYER. (4121)A

UNIVERSITY OF MANCHESTER

SCHOOL OF BIOLOGICAL SCIENCES

DEPARTMENT OF CELL AND STRUCTURAL BIOLOGY

CHAIR IN DEVELOPMENTAL BIOLOGY

The purpose of this new initiative is to complement existing strengths to make Manchester an internationally renowned centre of excellence in Developmental Biology. The principle criteria of appointment therefore, will be research excellence and/or research potential. Applications are invited from workers in all fields of developmental research: invertebrates, vertebrates, molecular, cellular or organismic aspects. The department already has major research projects in vertebrate: transcription factors and pattern formation, sex determination, implantation, gastrulation, craniofacial development, wound healing, growth factors, is well equipped for molecular, cellular and whole animal investigations, has a lively research atmosphere and excellent local and international collaborations. The person appointed will be responsible for teaching courses (with others) in Developmental Biology to medical, dental and science students, in which there is considerable scope for innovation. Opportunities exist to develop new teaching and research interests within the wide range of activities encompassed by the School of Biological Sciences. Informal enquiries to **Professor Mark W. J. Ferguson, Head of Department of Cell and Structural Biology (Tel: 061 275 6775).** Further particulars from the **Registrar, the University, Manchester M13 9PL** to whom applications (full curriculum vitae, names and addresses of three referees, and an outline research programme) should be sent by September 30th, 1990. *The University is an Equal Opportunity Employer.* (4125)A

Multidimensional Magnetic Resonance

Memorial Sloan-Kettering Cancer Center

Sloan-Kettering Institute, Memorial Sloan-Kettering Cancer Center is seeking an individual at the Assistant Member level to establish an independent program in multidimensional magnetic resonance studies of biologically active molecules. While the research activity will be independent, it is expected that the successful candidate will interact with other investigators at the Institute who are concerned with protein, nucelic acid and oligosaccharide structure and conformation. In support of these goals, the institute will make a significant investment in state-of-the-art magnetic resonance facilities and associated computational resources.

Applicants should provide a curriculum vitae and bibliography, three or more letters of recommendation from knowledgeable senior scientists in the field, and a thorough statement of research goals with some representative specific proposals.

All materials should be sent to:

Richard A. Rifkind, M.D.
Chairman, Sloan-Kettering Institute
Memorial Sloan-Kettering Cancer Centre
1275 York Avenue
New York, New York 10021

An Equal Opportunity/Affirmative Action Employer

(NW5525)A

THE AUSTRALIAN NATIONAL UNIVERSITY — The John Curtin School of Medical Research

DIVISION OF BIOCHEMISTRY AND MOLECULAR BIOLOGY

Research Fellow/Senior Research Fellow
Medical Molecular Biology

A position of research fellow/senior research fellow is available in the Medical Molecular Biology Group to collaborate with Professor Ian Young on the molecular biology of haemopoietic growth factors. Current research interests of the group include expression and structure-function studies on interleukin-5, molecular organisation and regulation of the interleukin-3 and interleukin-5 genes and gene targeting by homologous recombination.

Enquiries to Professor Young 61 6 249 0176 Ref JC 26.6.1

Postdoctoral Fellow/Research Fellow
Membrane Biochemistry

A position of postdoctoral fellow/research fellow is available in the Membrane Biochemistry Group to collaborate with Dr G. Cox in work on structure-function relationships in a number of membrane-associated complexes. Work is currently concentrated on the ATP synthase of *E. coli*; the two phosphate transport systems of *E. coli* and the human GABA-receptor. The experimental approaches used include gene cloning, DNA sequencing, mutagenesis, protein purification, in vitro membrane reconstitution and molecular model building. Structural studies are carried out in collaboration with NMR spectroscopists and X-ray crystallographers and functional studies with members of the Division of Neurosciences, JCSMR.

Enquiries to Dr Cox 61 6 249 2759 Ref JC 26.6.2

Postdoctoral Fellow/Research Fellow
Protein Biochemistry

Applications are sought from scientists with a PhD in biochemistry to work in collaboration with Dr Michael Denborough on the molecular basis of the anaesthetic complication, malignant hyperpyrexia. Experience in biochemistry, molecular biology and pharmacology is essential, and experience in phosphoinositide biochemistry would be a considerable advantage.

Enquiries to Dr Denborough 61 6 249 2207 Ref JC 26.6.3

Postdoctoral Fellow/Research Fellow
(Signal Transduction/Receptors) – Protein Biochemistry

Applications are invited from scientists with research interests within the general area of cell surface receptors and signal transduction in mammalian cells. The successful applicant would join a newly established Receptor-Cell signalling laboratory (Head: Dr P. D. Jeffrey) and would be given the opportunity to develop an independent research project on some aspect of biological signal transduction at the molecular level. Collaboration within the new laboratory or with other members of the Division would also be encouraged. Relevant aspects of research in the Division include protein-receptor interactions, signalling from growth factor receptors and ion channels and regulation of gene expression via signalling events.

Enquiries to Dr Jeffrey 61 6 249 3652 Ref JC 26.6.4

DIVISION OF CELL BIOLOGY

Postdoctoral Fellow/Research Fellow
(Immunologist/Cell Biologist) – Cell–Cell Interaction

Applications are invited from PhD graduates in immunology/cell biology for a position in the Cell–Cell Interaction Group to carry out research in collaboration with Dr C. R. Parish, leader of the group. The appointee will be involved in the identification and characterisation of cell adhesion molecules involved in lymphocyte recirculation and leukocyte-endothelial cell interactions. Familiarity with the biochemical analysis of cell membranes, cell culture techniques and the use of monoclonal antibodies is desirable.

Enquiries: Dr Parish 61 6 249 2604 Ref JC 26.6.5

Postdoctoral Fellow/Research Fellow
(Virologist/Immunologist) – Viral Engineering

Applications are invited from PhD graduates in Virology or Immunology for a position in the Viral Immunology Group to carry out research in collaboration with Professor R. V. Blanden, Group Leader and Dr A. Müllbacher. The appointee will be involved in the production of recombinant vaccinia, adeno, and alpha viruses encoding flavivirus proteins. The recombinants will be used to define areas of flavivirus proteins relevant to T lymphocyte epitopes, their relationship to B lymphocyte epitopes and their potential relevance in vaccines. Experience with the molecular virology of flaviviruses is highly desirable.

Enquiries: Professor Blanden 61 6 249 2596 Ref JC 26.6.6

Postdoctoral Fellow – Cell Transformation

A postdoctoral fellow is required to work in the Cell Transformation Group in collaboration with Dr A. W. Braithwaite. Work of the group concerns the roles of cellular and viral oncogenes (mainly Ela, T ag and P53) in control of gene expression, DNA replication, the cell growth cycle and differentiation. Preference will be given to candidates with experience in the molecular and cellular biology of cancer and cell transformation.

Enquiries: Dr Braithwaite 61 6 249 4480 Ref JC 26.6.7

DIVISION OF CLINICAL SCIENCES

Postdoctoral Fellow/Research Fellow —
Mucosal Inflammation and Cancer

A PhD graduate with expertise in free oxy-radicals is sought to collaborate with Professor Doe and Dr Baker in the Mucosal Inflammation and Cancer research program that focuses on the molecular basis of tissue injury in inflammation, especially oxy-radicals, serine proteases and cytokines. The research project will examine the role of inflammatory cell oxy-radicals and their products in the mediation of tissue injury in chronic mucosal inflammation, using an epithelial cell model and tissue from patients suffering from inflammatory bowel disease. Contemporary techniques will be used to study free radicals and to analyse intra and extracellular targets of free radical damage.

Enquiries: Professor Doe 61 6 249 2392 Ref JC 26.6.8

Postdoctoral Fellow — Developmental Haematology

A molecular cell biologist with an interest in cell differentiation is sought, to collaborate with Dr A. J. Hapel and Dr M. C. Fung in the Developmental Haematology Group on research in the area of signal transduction and/or gene regulation by haemopoietic cytokines. There is particular interest in discriminating at a molecular level signals essential for proliferation from those required to induce phenotypic changes (maturation) of bone marrow cells.

Enquiries: Dr Hapel 61 6 249 2381 Ref JC 26.6.9

DIVISION OF NEUROSCIENCE

Postdoctoral Fellow — Developmental Neurobiology

A postdoctoral fellow is required in the Developmental Neurobiology Group to collaborate with Dr C. Hill. The research will involve the biology of the developing autonomic nervous system, in particular the factors influencing the direction of axon outgrowth and the formulation of synapses and modulation of transmitter release. Such a person should have some experience in either *in vivo* or *in vitro* techniques pertinent to this area as well as some knowledge of biochemical or molecular biological methodology.

Enquiries: Dr Hill 61 6 249 4780 Ref JC 26.6.10

Postdoctoral Fellow/Research Fellow —
Neurophysiology OR Visual Neuroscience

An appointment is available, to be made either in the Neurophysiology Group or in the Visual Neuroscience Group,

— either to collaborate with Professor Redman in research on junctional mechanisms at central synapses, particularly in the hippocampus and spinal cord. Experience with *in vitro* slice preparation and whole cell recording techniques would be an advantage;

— or, to collaborate with Professor Levick in research on the spatial structure of pharmacological and electrical sensitivity in the dendritic fields of retinal ganglion cells *in vitro*. Previous experience in appropriate recording and micro-pharmacological techniques is desirable.

Enquiries: Professor, Redman, Neurophysiology 61 6 249 2602
Professor Levick, Visual Neuroscience 61 6 249 2525

Closing date: 10 August 1990 Ref JC 26.6.11

DIVISION OF CLINICAL SCIENCES

Mucosal Inflammation and Cancer Group

Postdoctoral Fellow/Research Fellow
(Molecular Geneticist/Molecular Biologist)

A PhD graduate molecular geneticist or biologist is sought to work on colon cancer genetics, especially familial non-polyposis colon cancer. The group comprises two cell biologists, a protein chemist and a molecular biologist, graduate students and technical support, and has a long-standing interest in colon epithelial cell transformation invasion and metastasis at the molecular level, and in colon cancer genetics.

Established experience in molecular genetics, preferably including random gene probing and candidate chromosome analysis is desirable. The successful applicant will work in a newly refurbished laboratory well equipped for molecular biology and molecular genetics, have ready access to a "state of the art" facility for oligonucleotide synthesis and automated DNA sequencing, and technical support. The project is funded by an external grant.

Enquiries: to Professor Doe — 61 6 249 2392.

Conditions

PDF: Appointment will be for 2 years initially, with the possibility of extension to a maximum of three years.

RF: Appointment will be for three years initially. If external funding permits, there is the possibility of extension, after review, to a maximum term of five years.

Closing date: 3 August 1990. Ref JC 26.6.12

Details of these positions may be obtained from the School Secretary JCSMR (Telephone 61 6-2492580) or by writing to the Registrar.

SALARY: Senior Research Fellow: A$45,729-A$54,255 p.a.; Research Fellow: A$33,163-A$43,096 p.a.; Postdoctoral Fellow Grade 1 (fixed point): A$28,792-A$32,762 p.a.

APPOINTMENT: Unless otherwise stated, Senior Research Fellow/Research Fellow up to three years, possibility of extension to five years; Postdoctoral Fellow up to two years, possibility of extension to three years.

APPLICATIONS should be submitted in duplicate to the **Registrar, the Australian National University, GPO Box 4, Canberra ACT 2601, Australia,** quoting reference number and including curriculum vitae, list of publications and names of at least three referees. The University reserves the right no to make an appointment or to appoint by invitation at any time. Further information is available from the Registrar, or from Appointments (38137), Association of Commonwealth Universities, 36 Gordon Square, London WC1H 0PF.

THE UNIVERSITY IS AN EQUAL OPPORTUNITY EMPLOYER (W7553)A

IMMUNO

AKTIENGESELLSCHAFT FÜR
CHEMISCH-MEDIZINISCHE PRODUKTE

Leading international manufacturer of biologics noted for pharmaceutical industrial research, due to further expansion, has immediate opening for

BIOCHEMIST

Candidates should have a Ph.D. in biochemistry, basic protein chemistry skills and relevant experience with isolaton, purification and characterization of proteins, protein sequencing, peptide mapping and amino acid analysis. Knowledge of plasminogen activators and clotting factors is preferred. Experience in carbohydrate-structure determination is a plus. Further, the scope of activities will include directing technicians in the development of assays, working with such techniques as HPLC, FPLC and conventional chromatographic techniques.

The position is a challenging opportunity for candidates looking for a responsible job with ample scope for development. Attractive salary plus benefits package is available. Compensation for re-location expenses will be provided.

Please send detailed résumé (including handwritten CV, remuneration required, date of availability) to REF 94/90

IMMUNO AG
Personnel Department
Dr. Pullez
A-1220 Vienna, Industriestraβe 67
Phone (43-1) 2300-2008

(W7548)A

PLANT/PATHOGEN MOLECULAR BIOLOGIST

The Department of Plant Pathology, University of California, Davis, invites applications for an 11-month (1 month vacation) tenure-track position, at the assistant professor level. The starting date is January 1, 1991, or as soon thereafter as the successful candidate is available.

The successful applicant will be expected to develop a research program dealing with the mechanisms which regulate the interaction between plants and pathogens at the molecular level. The focus can be on either the plant or the pathogen and can include molecular biology, cell biology, biochemistry, biophysics, genetics.

Participation in teaching of graduate and undergraduate courses and participation in seminar courses related to the applicant's expertise will be required. The applicant will be expected to supervise graduate students in plant pathology and related graduate groups, and may advise undergraduate students.

Applicants should have a Ph.D. in a discipline related to the study of molecular mechanisms regulating plant disease. Postdoctoral experience is highly desired but not required. Applicants should submit: (a) curriculum vitae, (b) list of published work and reprints, (c) a statement of teaching and research interests, (d) names and addresses of three references, and (e) undergraduate and graduate transcripts, if Ph.D. degree completed within the last five years, to:

Dr. David G. Gilchrist, Chairman, Search Committee
Department of Plant Pathology
University of California, Davis, CA 95616
Telephone: (916) 752-6938/0300

Application deadline: October 1, 1990.

THE UNIVERSITY OF CALIFORNIA IS AN AFFIRMATIVE ACTION/ EQUAL OPPORTUNITY EMPLOYER. (NW5522)A

UNIVERSITY OF QUEENSLAND

Brisbane, Australia

DEPARTMENT OF BOTANY

The Department is the leading provider in Queensland, and one of the leading providers in Australia, of undergraduate and postgraduate education in plant biology. Its goal is to address regionally and nationally important issues, in particular the conservation, management and, where appropriate, rehabilitation of valuable ecosystems through an understanding of process in plants and the dynamics of vegetation and evolutionary change. To this end, it will appoint two new staff, the Chair of Botany and a Lecturer (tenurable) in Marine Botany. It is planned to relocate the Department in 1993 into a refurbished building within the biology precinct.

CHAIR OF BOTANY

The appointee will be a distinguished experimentalist in the areas of ecology and evolution of tropical terrestrial plants and plant communities, who will provide academic leadership to the Department as a whole, and enhance the strengths of the different areas of teaching and research activity.

A particular responsibility will be to provide leadership to the ecology and physiology group, which currently includes 5 academic staff, and is concerned primarily with the understanding of processes within tropical and subtropical forests as a basis for their conservation and management. A major research project of the group is located in a southern Queensland rainforest, and joint research programmes link the Department with several state and federal agencies, with the Institute of Rainforest Studies in Cairns, and with overseas researchers. The position also allows exciting opportunities for the development of collaborative research programmes within the University under the auspices of a Centre for Conservation Biology which will provide a focus for the research of whole-organism biologists.

The appointee will be expected to assume the Headship of the Department for a period of 5 years in the first instance.

Salary: A$67,812 per annum. (A salary loading for Headship applies).

Reference No: 33590 Closing date: **30 August 1990**

LECTURER IN BOTANY
(Tenurable)

The successful applicant will have an established record and an active research interest in marine botany, preferably in subtropical or tropical areas. The appointee will be expected to teach in botany, with particular responsibility for undergraduate subjects and postgraduate supervision in marine botany, to conduct research in marine botany, and to contribute to the scholarly activities of the Department. Outstanding opportunities exist for collaborative teaching in the School of Marine Science within the University, and for research at Heron Island on the Great Barrier Reef, in coastal southern Queensland, and in experimental marine and freshwater aquaculture facilities in Brisbane and nearby.

Salary: A$33,163 – A$43,096 per annum.

Reference No: 33690 Closing date: **30 August 1990**

Application forms and further details concerning method of application are available from Appointments (38145), Association of Commonwealth Universities, 36 Gordon Square, London WC1H 0PF.

Equal Opportunity in Employment is University Policy (W7532)A

UNIVERSITY OF DUNDEE

DEPARTMENT OF BIOLOGICAL SCIENCES

LECTURESHIP IN GENETICS

Applications are invited from outstanding candidates in the field of molecular genetics for a lectureship which is available from 1 October 1990.

Candidates with research experience and interests in the molecular genetics of eukaryotes are especially encouraged to apply, although applications in prokaryotic genetics will also be welcomed.

The salary is negotiable and will be within the Non-clinical Lecturer scale (Grade A: £12,086-£16,755; Grade B: £17,455-£22,311). The appointee will be encouraged to rapidly establish a research programme in an active and well-equipped department and to teach at junior and senior levels in BSc Hons courses in biological sciences.

Further information can be obtained by telephoning 0382 23181 ext. 4767.

Applications in writing with curriculum vitae (6 copies or, if posted overseas, one copy in a format suitable for photocopying) and the names and addresses of three referees should be sent as soon as possible to the **Personnel Office, The University, Dundee, DD1 4HN.** *Please quote reference EST/33/90/N.* (4159)A

National Institute for Biological Standards and Control

Head of Division of Haematology

Applications are invited for the position of Head of the Division of Haematology at NIBSC which will become vacant in October 1990 upon the retirement of the present post-holder, Dr. D. P. Thomas.

The division takes a leading role both nationally and internationally in the standardization and control of therapeutic products used in the field of haemostasis and thrombosis; these functions are supported by an active research programme. Current work is focused on clotting factor concentrates, anticoagulants and thrombolytic drugs and increasingly involves evaluation of products derived by recombinant DNA methods. The Institute liaises with the UK Blood Transfusion Service and plans to strengthen its activities in the field of transfusion medicine, including work on immunohaematology. It is anticipated that this development will be further strengthened and co-ordinated by the new Head of Division.

The Division provides advice to the Medicines Control Agency and the Committee on Safety of Medicines. It collaborates closely with the World Health Organization in the establishment of International Standards in the field of blood products and is increasingly active in the European Community. The Institute operates a multi-disciplinary grouping of collaborating scientific divisions, which are supported by a range of service and administrative units.

Candidates should have medical and/or scientific qualifications with previous experience in haematology and/or transfusion medicine as well as an excellent track record in an appropriate field of scientific research.

Salary will be on an appropriate scale up to £34,203 per annum (under review); however, salary may be negotiable beyond this point for exceptionally well qualified and experienced candidates.

Informal enquiries may be made to the Director, Dr. G. C. Schild.

Further details are available from the Personnel Department.

Applications, with full curriculum vitae, should be made to the Director, NIBSC, Blanche Lane, South Mimms, Potters Bar, Hertfordshire EN6 3QG. Tel: 0707 54753.
Please quote reference number: BL/032.

Closing date for applications is: 3rd August 1990. (4120)A

University of Cambridge
Department of Clinical Biochemistry

Research Assistant/Associate

A position is available for 2 years initially from September 1990 on a project involving the isolation and molecular cloning of pancreatic islet proteins which are targets of cell-mediated autoimmune attack in diabetes (see Roep et al., (1990), Nature 345: 632). The applicant should have academic qualifications in either immunology, biochemistry or molecular biology. Experience with protein purification, recombinant DNA techniques and mammalian tissue culture would be a considerable advantage.

The appointment will be on the Cambridge unestablished research workers salary scale up of £14,744 p.a. depending upon age and experience. Applications with a full curriculum vitae and the names of at least two referees should be sent to **Dr. J. C. Hutton, Department of Clinical Biochemistry, University of Cambridge, Addenbrooke's Hospital, Hills Road, Cambridge, CB2 2QR, U.K.** from whom further details can be obtained. Tel. (0223) 336790, Fax. (0223) 216862.

The University follows an equal opportunities policy. (4133)A

UNIVERSITÄT ULM

In der Abteilung Vergleichende Neurobiologie der Universität Ulm ist eine

Postdoktorandenstelle (BAT IIa) Neuro-/Zellbiologie

für 2 Jahre im Projekt über Hormonregulation im Gehirn und Thymus zu besetzen.

Voraussetzung: Erfahrung mit Immuncytochemie und/oder in-situ-Hybridisierung.

Schwerbehinderte werden bei entsprechender Eignung vorrangig eingestellt.

Bewerbungen an: Prof. Dr. G. Ehret, Abteilung Vergleichende Neurobiologie, Universität Ulm, Postfach 40 66, D-7900 Ulm. (W7535)A

EXECUTIVE DIRECTOR

The Center for Field Research-Earthwatch is seeking a senior scientist to administer our grants program in support of field research in the physical, biological, and social sciences. The 1991 program will provide over $3 million for 135 projects in 60 countries and 20 states. The Executive Director's responsibilities include overall program analysis, advising program officers, representing CFR-Earthwatch at major conferences, development of education and scholarship programs, and procurement of external research support. Excellent organizational and personnel skills are required to work with other managers in field operations, publications, development, and finance. Applications should be received by 1 August 1990. Send CV and three references to **Dr. R. D. Estes, Acting Director, Center for Field Research, Earthwatch, P.O. Box 403, Watertown, MA 02172.** (NW5521)A

Elsevier Science Publishers (Japan) 正社員募集

“日本の科学技術情報を海外へ”

編集部スタッフ：工学、理学系１名（学部卒業者（男女））

オランダに本社を置く世界有数の学術出版社グループです。日本での事業規模拡大に伴い編集スタッフを募集します。

資　格：１　25～30歳
　　　　２　理工系の学部卒業以上の学歴／材料、物理、情報の分野専攻の方を希望
　　　　３　英語力（特に読み書き）必要
　　　　４　出版業務経験有の方歓迎
待　遇：完全週休２日制／社会保険完備
応　募：英文・和文履歴書（写真貼付）を各１通郵送下さい。

(W7543)A

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Royal Postgraduate Medical School
(University of London)

(Hammersmith Hospital)
DEPARTMENT OF VIROLOGY

Lecturer or Senior Lecturer

Applications are invited for a permanent (tenure-track) position in the Department of Virology. Appointment will be made either at Lecturer or Senior Lecturer level depending upon the qualifications and expertise of the applicant. Medically qualified persons may be appointed to an Honorary Consultant post in the Hammersmith Hospital. The ideal candidate will have a strong interest in basic research, some experience in molecular and cellular biology, and the desire to establish an independent scientific research programme in an area of medical-related virology. There is a teaching commitment in the Department and some experience in lecturing and training PhD students will be an advantage. The current interest in the Department centres around herpes — and oncogenic viruses, but applicants from other areas of virology are also welcomed. The Department is modern and well-equipped, and expansion is planned within 6-9 months to include a central service in molecular biology and tissue culture. Appointment may be made subject to a review or probationary period and salary will be appropriate to the qualifications and experience of the appointee.

Informal enquiries to Professor Beverly Griffin, Department of Virology, telephone 081-740 3248.

Letters of application, together with a full c.v. (7 copies) plus the name and addresses of 3 referees should be sent to the **Personnel Officer, Royal Postgraduate Medical School, Ducane Road, London W12 0NN (tel: 081 740 3204)** from whom further particulars are available. Ref: AV02

Closing date: 31 August 1990.

Laboratory Manager/MLSO 3/4

The Department of Virology at the RPMS has grown over the last few years to the point where it becomes desirable to establish a new position of **Laboratory Manager**. It is hoped to appoint at a senior level someone with a strong interest in laboratory science and management skills. There is considerable flexibility in the post, but preference will be given to a person with an interest and experience in microbiology, seeking a career that involves decision taking and responsibility. The department has an active interest in both clinical diagnosis and research into viruses associated with human disease, especially oncology. For details, contact the **Personnel Office, Royal Postgraduate Medical School, 150 Ducane Road, London W12 0NN or telephone 081-740 3204.** Salary scale, up to £17304 (+ £1580 London Allowance) MLSO 3 or exceptionally MLSO 4, depending upon qualifications and experience. The holder of this post will be directly responsible to the Head of the Department.

Closing Date: 31 July 1990.

(4134)A

MOREDUN RESEARCH INSTITUTE

Higher and Senior Scientific Officer (2 posts)

(Three-year Appointments)

Applications are invited for the following 2 posts at Senior Scientific Officer (SSO) or Higher Scientific Officer (HSO) grades.

Post 1 (readvertised). Ref: MRI 1.

A protein biochemist or chemist, preferably with experience of isolating and characterising proteins from membranes and other subcellular fractions, is required to join a multidisciplinary team investigating the immunogenicity of proteins/peptides derived from the protozoan parasite *Toxoplasma gondii.*

Post 2. Ref: ADRA 1.

A biochemist/immunologist with experience in protein chemistry to join a group studying the role of serine proteases and their inhibitors (serpins) in the pathogenesis of disease. The successful applicant would study the role of neutrophil serine proteases and of serpins in the pathogenesis of equine endometritis (Funded by the Horse Race Betting Levy Board).

Applicants must have a 1st or upper 2nd honours degree in biological sciences or equivalent qualification. A PhD or postgraduate experience relevant to the respective duties will be advantageous.

Salary scales:- HSO £10,678-14,909 SSO £13,254-18,864.

Applications, in triplicate, with full CV and the names and addresses of two referees, should be sent to the **Secretary, Moredun Research Institute, 408 Gilmerton Road, Edinburgh EH17 7JH.**

Informal enquiries to Tel: (031) 664 3262 (Dr. H.R.P. Miller or Dr. D. Baird). Fax: (031) 664 8001. (4157)A

UNIVERSITY OF GLASGOW
Wellcome Laboratories for Experimental Parasitology
RESEARCH ASSISTANT

Applications are invited for the above post to work on the secreted antigens an proteinases of nematode parasites. The project is funded by the Wellcome Trus for three years beginning October 1990. The successful applicant will join laboratory working on a variety of projects on the immunochemistry a biochemistry of parasitic nematodes. Applicants should have a good honour degree in molecular biology, biochemistry, or related fields.

The post is available from October 1990, and carries with it the opportunity fo registration for a higher degree. Starting salary will be on the University RA 1 scale (stimulated by 2 increments) from £12,792 and appropriate annual increments thereafter.

Applications including curriculum vitae and the names, addresses and telephone numbers of at least two adademic referees should be sent as soon as possible to **Dr Malcolm Kennedy, Wellcome Laboratories for Experimental Parasitology, University of Glasgow, Bearsden, Glasgow G61 1HQ** to whom informal enquiries can be made on 041-339 8855 Extn 5819 or 6968. (4137)A

HERMANN-VON-HELMHOLTZ-PROGRAMM

Der Bundesminister für Forschung und Technologie gewährt im Rahmen des Förderprogramms

NEUROBIOLOGIE

besonders qualifizierten deutschen Nachwuchswissenschaftlern, die nach einem mindestens einjährigen Forschungsaufenthalt im Ausland nach Deutschland zurückkehren möchten, zeitlich befristete Forschungsbeihilfen. Die Forschungsbeihilfe wird in der Regel für 2 Jahre vergeben. Sie umfaßt eine Stelle vergleichbar BAT IIa/Ib und einen Pauschalbetrag für Sachmittel. Die Vergabe der Forschungsbeihilfen erfolgt auf Grund eines Auswahlverfahrens nach wissenschaftlichen Gesichtspunkten. Ein Merkblatt über Forschungsbeihilfen und Antragsformulare können bei der zuständigen Geschäftsstelle.

Naturwissenschaftliches und Medizinisches Institut
an der Universität Tübingen in Reutlingen (NMI)
Gustav-Werner-Str. 3, 7410 Reutlingen

angefordert werden. Forschungsbeihilfen werden erstmalig ab 01.01.91 vergeben. (W7541)A

ASSISTANT PROFESSOR

Department of Structure and Chemistry of Biopolymers
Bayreuth University

Two full-time positions on the assistant professor level are available immediately in the field of NMR of biomolecules. Both positions are for 3 years initially (renewable), one of them is tenure track. Areas of interest include structure determination of proteins (kinases) and synthetic peptides. The department is planning to have a 600 MHz spectrometer installed in early 1991. Adequate computing and biochemical facilities are available. The successful applicants are expected to have postdoctoral training in the field of structural NMR and/or protein chemistry.

Salaries are according to the German government salary scale and depend on age and marital status.

Please forward applications including a cv and a list of publications to **Professor Paul Rösch, Lehrstuhl für Struktur und Chemie der Biopolymere, Universität Bayreuth, Postfach 10 12 51, D-8580 Bayreuth, FRG.** (W7544)A

Imperial College of Science, Technology and Medicine
Department of Biochemistry

Molecular Genetics of Neural Development

MRC-funded position available for a post-doctoral research assistant to study the molecular basis for developmental decision making processes during Drosophila neural development with Dr David Hartley. By a combination of genetic, molecular and cell biological techniques, we hope to understand the dynamic interplay between the cell surface and the nucleus which permits the developing nerve cells to distinguish and react to extrinsic cues. The research assistant will be involved in the identification, phenotypic characterization and eventual cloning of genes which regulate developmental choices of incipient nerve cells.

Salary on IA scale £10458-£16665 (under review) plus £1767 London Allowance.

Experience in genetic and/or molecular biological and/or cell biological techniques is an advantage. Applicants should send a CV with names, addresses and phone numbers of two referees, to **Dr D Hartley, Department of Biochemistry, Imperial College of Science, Technology and Medicine, London SW7 2AZ.** (4132)A

International Rice Research Institute

Deputy Director General for Research

The International Rice Research Institute seeks a Deputy Director General for Research. The position becomes vacant in May 1991.

IRRI IRRI is an international, non-profit, agricultural research institute funded by some 30 donor nations and organizations. Its goal is the enhancement of rice production and sustainability to benefit the world's 3 billion people who are dependent on rice for their livelihood and as their basic food.

Responsibilities

- Provide leadership in the establishment of research priorities and the application of innovative scientific approaches to address problems of increasing the productivity and sustainability of rice-based systems.
- Direct IRRI's multi-disciplinary research programs and discipline-oriented research divisions.
- Enhance ties with advanced agricultural research institutes.

Qualifications

- PhD, with extensive experience and a record of high quality agricultural research.
- Sound knowledge of and experience with agriculture in the developing world.
- Vision and research leadership, ability to communicate with and inspire high performance from scientists of different nationalities and disciplines.
- Proven record in research management and administration.
- Fluency in written and spoken English.

Benefits Salary and perquisites are competitive with those of similar international institutions. The Deputy Director General for Research will live at IRRI headquarters in Los Bañoc, Laguna, 60 km south of Manila, Philippines.

Interviews Interviews of short-listed applications will commence in September 1990.

Apply to Applications with full documentation, including the names and addresses of three referees, and names of nominees should be addressed to Dr. Klaus J. Lampe, Director General, IRRI, PO Box 933, 1099 Manila, Philippines. Phone (63) 2-818-1926; FAX (63) 2-818-2087.

(W7530)A

UNIVERSITY COLLEGE
& MIDDLESEX
SCHOOL OF MEDICINE

Professor and Head of the Department of Medicine

Applications are invited for an established Chair of Medicine with the appointee assuming the headship of the Department of Medicine on the retirement of Professor S.J. Semple in September 1991.

Applicants should have a strong commitment to undergraduate and postgraduate teaching and a personal record of distinction in research. The Department of Medicine has academic activities in association with The Middlesex, University College and Whittington Hospitals; no specific speciality interest of applicants is being sought.

Applications including a full curriculum vitae and the names of three referees should be sent within 4 weeks of the appearance of this advertisement to the Provost, University College London, Gower Street, London WC1E 8BT. Further particulars about the post are available from the **Dean, University College and Middlesex School of Medicine, Riding House Street, London W1P 7PN** (Tel: 071-380 9306). *Equal Opportunities Employer.* (4119)A

UCL

Nestlé

Would you like to use your scientific background within an international food company? Are you looking for a position where you can use your negotiating talents and your sense of diplomacy?

Our legislation Department is seeking a

BIOTECHNOLOGIST (MOLECULAR BIOLOGY AND GENETICS)

or

ENGINEER IN ENVIRONMENTAL SCIENCES

or

FOOD TECHNOLOGIST

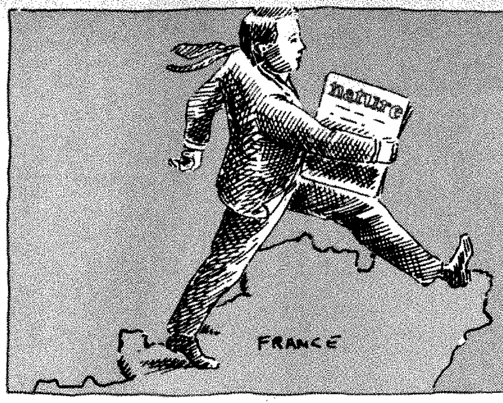
who will be responsible for

- the supervision of the development of food legislation at a national and international level in the above fields
- active participation in the preparation of new legislative texts
- assistance to the international headquarters in Vevey and to operational companies abroad.

For this position we require:

- University degree in biotechnology, environmental sciences or food technology
- Several years experience in the above fields
- Knowledge of French, English and German
- Good negotiating and communication skills.

Kindly forward your application, accompanied by the usual documents to: **Mr. E. Brambilla, Personnel Department, Nestlé, 1800 Vevey.** (W7558)A



SCIENCE IN FRANCE

Au coeur de la santé

ROUSSEL-UCLAF, Groupe Pharmaceutique et Agrochimique Français à vocation internationale intervient dans 3 secteurs d'activité : Santé humaine, animale et végétale.

Dans le cadre de l'activité phytosanitaire du Groupe et pour notre centre de Recherche de Biologie Appliquée situé à Marseille, nous recherchons un :

ENTOMOLOGISTE

Vous êtes Ingénieur Agronome ou Docteur es Sciences, spécialisé en entomologie, les relations "structures chimiques/activités biologiques" vous passionnent et vous avez le goût de la recherche et du travail en équipe.

Nous vous proposons de contribuer à la découverte de nouvelles molécules à vocation insecticide, en concevant et réalisant des études spécifiques : essais, tests biologiques, études de mécanismes d'action.

Vous serez en liaison avec les autres services du centre de recherche, notamment synthèse chimique et développement.

Merci d'adresser lettre manuscrite, CV et prétentions, s/réf. 114/N, ROUSSEL UCLAF Direction de l'Encadrement et de la Formation - 35, bd des Invalides, 75007 PARIS

ROUSSEL UCLAF

(W7540)A

INSTITUT COCHIN DE ICGM GENETIQUE MOLECULAIRE

INSERM CNRS ANRS FACULTE DE MEDECINE COCHIN

ICGM PRACTICAL COURSES

EPITOPE MAPPING USING BACTERIAL EXPRESSION VECTORS

PARIS September 10-21 1990

Organizers:

Keith STANLEY (Sydney)
George BANTING (Bristol)
Richard BENAROUS (Paris)

The course will be based on the epitope mapping technology developed at EMBL by Keith Stanley and coll. (EMBO J. (1987) 6, 1951-1957).

Course objectives:

1—**Construction of epitope libraries from participant's cDNA**. This will be fragmented and cloned into pEX627.

2—**Screening of the epitope library** and colony purification. Participants should bring their own polyclonal serum or monoclonal ascites.

3—**Sequencing of positive clones.**

Maximum number of participants: 20 Course fees: 9000 french francs, lunches included but not accommodations.

Registration deadline **10 August.** Application forms are available from: **Dr Richard Benarous ICGM 22 rue Mechain 75014 Paris France.** Telephone: 33 (1) 40 46 04 11. Fax: 33 (1) 40 51 77 49. (W7539)C

BIOMAT '90
POLYMERS AND IMMOBILIZED CELLS OR BIOMOLECULES
Fundamentals and Applications
December 12-14 1990
BORDEAUX, FRANCE

The general objective of this meeting is to enlarge the usual biomaterial area, ie design, processing, characterization, application for medical devices and functional assistance, to more general aspects of interactions of biomaterials with biological systems, and to various issues of related studies. The following list of topics correspond to this intention.

Hybrid biomaterials; Bio artificial organs; Bioactive polymers; Drug delivery systems; Biosensors; Affinity matrixes; Biotechnology; Biological reagents.

Applications to:- **M. Charles BAQUEY, BIOMAT '90, INSERM U 306, Université de Bordeaux II, 146, rue Léo Saignat, 33076 BORDEAUX Cedex.** Tel: (33) 56 93 12 711. Fax: (33) 56 24 94 65. (W7525)C

X e Colloque de la Société Française de Biologie du Développement (SFBD)

5-8 septembre 1990 à Banyuls-sur-mer.

Thèmes: Cycle cellulaire et Développement précoce; Génétique et Neurogenèse; Communications libres.

Information et Inscription: Observatoire Océanologique (Laboratoire Arago), H-J. Marthy, 66650 Banyuls-sur-mer. Tél. 68 88 00 40; Fax: 68 88 16 99. (W7523)C

Important private Medical Research Center in the Paris area, member of one of the leading pharmaceutical companies in France is hiring for its

DEPARTMENT OF CELLULAR AND MOLECULAR BIOLOGY

Two Senior Researchers in Biology/Biochemistry

(PhD. or post-doctoral level)

The successful candidates have a good experience in mRNA isolation, protein purification, Northern and Western blot and are creative and independently working individuals. They are interested in the isolation and characterization of transport proteins or the expression of oncogenes.

Please submit CV to Média-System, 6 impasse des Deux Cousins, 75849 Paris Cedex 17 France. Please quote reference 2044 on the envelope.

MEDIA-SYSTEM

(W7557)A

Eighth National Meeting of the French Society for Mass Spectrometry

19-21 September 1990

University of Nice - Sophia Antipolis, Nice, France

This meeting will be organized by the FT-ICR Group at the University of Nice Sophia Antipolis under the auspices of the French Society for Mass Spectrometry (SESM) and the Mass Spectrometry Group of the French Society for Chemistry (SFC) in connection with the CNRS and the GAMS. The scientific program will cover all aspects of Mass Spectrometry. Plenary and keynote lectures will be given by invited speakers covering the latest advances in the field, in particular:

- Hyphenated techniques: TG-MS, ICP-MS, SFC-MS.
- Ionization methods: laser, ion spray, API, FAB and FT-ICR.
- Instrumentation: ion trapping techniques, TOF.

Applications of Mass Spectrometry to Biochemistry, Flavours and Fragrances and Geochronology will be among the topics.

Further details are available from:

Dr. P.-C. MARIA, GROUPE FT-ICR, Laboratoire de Chimie Physique Organique, Université de Nice – Sophia Antipolis, Parc Valrose, 06034 NICE Cédex, France. Tel: (33) 93 52 98 52. FAX: (33) 93 52 99 19. (W7545)C

UNIVERSITÉ DE BESANÇON

Department of Biochemistry and Molecular Biology

The Science Faculty of Besançon (France) invites applications for a position of Professor of Molecular Biology

The professor will have to teach Molecular Biology and will have to conduct research concerning the modification of monoclonal antibodies by using gene manipulation techniques. This research programm is supported by the CNRS.

Applicants should have a very good record of research achievement in Molecular Biology, and are expected to be able to teach in French. The post will be available in October 1991.

Applications including a C.V., a list of publications, the names of two referees and two reprints of the three most important publications should be submitted to: **Professor Y. Gaudemer, Laboratoire de Biochimie – Biologie Moléculaire, UFR Sciences et Techniques, Route de Gray, 25030 Besançon Cedex – France.** Phone number: 81.66.62.44.

(W7521)A

The Research Division in RHEUMATOLOGY of an important pharmaceutical research center located in **PARIS suburb**

is looking for

A research worker in cell culture and bone tissue

The profile : a graduate from university (third cycle) if possible and Post-Doctorate having good knowledge in bone biochemistry.

Interested by the creation of knew molecules, particularly for the treatment of osteoporosis.

He will take in charge the culture unit within a young team of research working on bone metabolism.

Thanks for sending a manuscript letter, C.V. and claims to *PUBLIVAL/7002* - 27 Route des Gardes, 92190 MEUDON (FRANCE).

(W7538)A

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Leader mondial des Systèmes de Modélisation Moléculaire, grâce à son logiciel Sybyl

recherche un
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pour renforcer l'équipe Tripos basée à Palaiseau (91).

Chercheur expérimenté dans le domaine de la Modélisation Moléculaire, vous souhaitez travailler au sein d'une équipe dynamique en liaison avec nos autres centres européens et notre siège aux Etats-Unis.
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Adresser CV et prétentions à **Evans & Sutherland, 3, Allée des Garays 91124 Palaiseau Cédex** ou contacter S.Briggs au 33.1.69.20.46.47. (W7547)A

NATO Advanced Research Workshop

GENOME ORGANIZATION AND EVOLUTION

Spetsai, Greece
15-19 September, 1990

A NATO Advanced Research Workshop on Genome Organization and Evolution will take place in the island of Spetsai, Greece, between September 15-19, 1990.

A partial list of speakers includes: W. Arber (Basel), G. Bernardi (Paris), T. Bettecken (Würzburg), R. J. Britten (Corona del Mar), M. Burmeister (San Francisco), G. Dover (Cambridge, U.K.), B. Dutrillaux (Paris), W. Fitch (Irvine), C. Gautier (Lyon), J.H. Gillespie (Davis), M. Gouy (Lyon), G. Holmquist (Duarte), T. Ikemura (Mishima), T. Jukes (Berkeley), J. Jurka (Palo Alto), W.-H. Li (Houston), G. Macaya (Paris), D. Mouchiroud (Lyon), J.-C. Mounolou (Orsay), M. Nei (Houston), T. Ohta (Mishima), E. Olmo (Naples), G. Roizès (Montpellier), C. Saccone (Bari), M. Schmid (Würzburg), V. Sgaramella (Pavia), P. Sharp (Dublin), V. Stingo (Naples), A.T. Sumner (Edinburgh), E. Zuckerkandl (Palo Alto), and H. Zuber (Zürich).

Persons interested in participating in this Workshop should send a brief curriculum vitae, a list of publications, a statement on their research interests and, if they so wish, an abstract of their oral or poster presentation to **G. Bernardi, Laboratoire de Génétique Moléculaire, Institut Jacques Monod, 2 Place Jussieu, 75005 Paris, France, Fax 33-1 46 33 23 05, before August 15.** Accepted participants will have to take care of their travel expenses and living expenses (the latter amount to about 150 USD). A number of Fellowships to defray such costs will be available for young participants.

(W7556)V

USE THE FAX

- to save time and for your added convenience you can contact us by fax.
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SEILLAC 1989

SECOND INTERNATIONAL CONFERENCE ON BISPECIFIC ANTIBODIES AND TARGETED CELLULAR CYTOTOXICITY

Seillac, France. October 9-13, 1990.

The first International Conference on Biospecific Antibodies was held in Annapolis, MD, on October 22-25, 1989. The studies which were presented there helped to greatly increase our understanding of the cytotoxic potential of effector cells, most notably T cells and macrophages, which have been triggered by bifunctional antibodies.

The 2nd International Conference will further our objective to bring together basic researchers, clinicians, and representatives of commercial organizations to present their most recent results using bispecific antibodies for medical and research purposes. The format of the meeting will be similar to that followed in the Annapolis conference: there will be oral presentations by selected speakers as well as poster sessions. This meeting will provide a solid base of information useful for the design of bispecific antibodies to direct the cytotoxic potential of monocytes, PMN, NK cells, and T cells for therapy of cancer and infectious disease. The second conference will also present an excellent opportunity for participants to review all ongoing clinical protocols using targeted cytotoxic cells.

As in last year's meeting, the 2nd International Conference will accommodate a small number of registrants — approximately 120 people. Participants will include teams of established investigators as well as scientists who have recently entered the field.

The 2nd International Conference will take place at Le Château de Seillac, located in the Loire Valley. This area is well known for its rich historic heritage, as well as for some of the finest wines of France. All participants will be housed in the Château or in one of the condominiums located on the castle grounds. Lodging will be arranged by the Organizing Committee at Centre National de Transfusion Sanguine (CNTS) PARIS.

Invited Speakers

Dr. David Segal	USA	Dr. Hermann Waldmann	UK
Dr. Wolf Fridman	France	Dr. Kris Thielmanns	Belgium
Dr. A. M. Schmitt	France	Dr. Edward Ball	USA
Dr. Martin Glennie	UK	Dr. Michael Fanger	USA
Dr. Jeffrey Ledbetter	USA	Dr. Jacques Bartholeyns	France
Dr. Pierre Henkart	USA	Dr. Steven Chamow	USA
Dr. Louis Weiner	USA	Dr. John Donohue	USA
Dr. Reinder Bolhuis	Netherlands	Dr. Sven Warnaar	Netherlands
Dr. Ko Okumura	Japan	Dr. Maria Colnaghi	Italy
Dr. Jan Van de Winkel	Netherlands	Dr. Clemens Sorg	Germany

Organizing and Program Committee

M. Fanger (Dartmouth University USA), J. L. Romet-Lemonne (CNTS France), D. Segal (NIH USA)

For any information

Centre National de Transfusion Sanguine
3, avenue des Tropiques BP 100 91943 LES ULIS CEDEX France
Conference Secretariat: Mrs. M. Furgollo
Tel.: (1) 69.07.78.71 Fax: (1) 69.28.54.28 Telex: 602729F

(W7546)C

MOLECULAR BIOLOGIST

Neuroscience CNRS/FRANCE

Post-doctoral scientist to join a pluridisciplinary group (molecular neurobiologists and electrophysiologists) working on excitatory amino acid receptors. The laboratory is located in a large institute of neurosciences. The institute has a staff of 200 neurobiologists and is part of a large and pleasant campus just out of Paris. Salary according to experience. Further information can be obtained informally by contacting **Jean Rossier** or **Lia Prado de Carvalho at 33 (1) 69.82.34.36, Laboratoire de Physiologie Nerveuse, CNRS, 91198 Gif sur Yvette, CEDEX, FRANCE.** (W7533)A

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1st HFSP-Workshop:
Regulatory mechanisms of DNA replication — 17-23 March 1991 — Les Arcs 2000 — France — cosponsorship FEBS (See Nature 7 June, page 27). (W7526)V

Au cœur de la Santé

ROUSSEL UCLAF, groupe pharmaceutique et agrochimique français à vocation internationale intervient dans trois secteurs d'activité : Santé humaine, végétale et animale.

Présentes dans le monde entier, nos équipes de recherche, développement, fabrication, marketing et vente démontrent chaque jour leur compétence et leur volonté de progresser.

Dans le cadre de notre développement, nous recherchons un

CHEF DE LABORATOIRE EN PHARMACOLOGIE

Au sein du département «endocrinologie» vous serez chargé d'animer et de coordonner une équipe de recherche fondamentale de 4 personnes opérant dans les domaines suivants :

- *Recherche d'applications thérapeutiques sur des anti-hormones dans le domaine des stéroïdes.*
- *Mise au point de nouveaux modèles d'expérimentation.*
- *Rédaction et présentation des dossiers pharmacologiques.*
- *Echanges pluridisciplinaires au sein du groupe et participation aux congrès traitant ce sujet.*

Docteur en pharmacologie avec de préférence une spécialité en endocrinologie, vous avez 3 à 5 ans d'expérience dans le domaine de la recherche.

La pratique de l'anglais est impérative.
Le poste est basé à Romainville (93).

Si cette opportunité vous intéresse, nous vous remercions d'adresser au plus tôt lettre manuscrite, CV et prétentions sous référence 122G à SWITCH 40, rue Amelot 75011 PARIS qui traitera votre dossier en toute confidentialité.

ROUSSEL UCLAF

(W7536)A

AFFLUENTS

INSERM PARIS

Neurodegenerative Disease Unit

3 Years Postdoctoral Fellowship (Electron Microscopist)

Applications are invited for the post of full time researcher to support a group of 35 persons working on the mechanism of cell death in Parkinson's and Alzheimer's disease.

The postholder will be responsible for the management of the electron microscope and ultramicrotome. The applicant should be able to conduct a research project and teach EM techniques to the other members of the team.

Experience in thin sectioning of biological material, and immunohistochemistry techniques at the EM level is required. In addition, experience in managing EM facilities and image analysis is desirable. Knowledge of the central nervous system ultrastructure would be an advantage.

Appointment will be made for a period of 3 years starting as soon as possible. Salary will depend on experience.

Applications, together with C.V., list of publications and names and addresses of two referees, should be sent to: *Dr. AGID, INSERM U289, Hôpital de la Salpêtrière, 47 bd de l'Hôpital, 75013 Paris, France or Fax (33) 1 44 24 36 58.*

(W7524)E

NEW ZEALAND DEPARTMENT OF SCIENTIFIC AND INDUSTRIAL RESEARCH

DSIR Chemistry

Research Associates New Zealand

DSIR Chemistry is a large division of a New Zealand Government Department located at Lower Hutt, undertaking basic and applied research as well as commercial contracts.

Applied Protein Chemistry

Functional modification of proteins and/or the application of enzymes as catalysts as part of an Industrial Biocatalysis programme. Contact: Dr. R H Furneaux.

Atmospheric Trace Gases and Aerosols

Atmospheric trace gas or aerosol analysis as part of the clean-air monitoring programme currently being run at Baring Head, New Zealand, in collaboration with the DSIR Institute of Nuclear Sciences and the New Zealand Meteorological Service. Contact Dr. A L Dick.

Ceramic Coatings

Ceramic coatings involving sol-gel or metal-organic ceramic precursor synthesis. The programme complements existing programmes in Engineering Ceramic synthesis, fabrication and characterisation. Contact Dr. I W M Brown.

Dioxins in Pulp/Paper

Investigate the sources of, and mechanistic routes to, dioxin formation during pulp bleaching and paper production. Contact Dr. D Hannah.

Polysaccharide Structure

Development of new techniques and their application to commercially interesting algal, animal, bacterial and plant polysaccharides from New Zealand agricultural resources. Contact Dr. R H Furneaux.

Applicants for these positions should have a PhD and relevant experience. The positions are for one year, renewable by mutual agreement. Salary will be in the range of NZ$30,000-40,000 per annum, with assistance for travel and relocation.

Enquiries should be made to the contact named above,
DSIR Chemistry,
Private Bag, Petone,
New Zealand,
Telephone 64 4 666-919, facsimile 64 4 694-500.

(W7519)A

THE UNIVERSITY OF LEEDS
Department of Biochemistry and
Molecular Biology

CHAIR OF BIOCHEMISTRY

Applications are invited for the established Chair of Biochemistry which will become available on the retirement of Professor Donald S Robinson in September 1990. This prestigious Chair will be held in major new department, formed by the merger of the present separat Departments of Biochemistry and Biophysics. Applicants who hav an international scientific reputation in any area of biochemistry o molecular biology will be considered. The salary will be within the non-clinical professorial range.

Further particulars may be obtained from the **Registrar, The University, Leeds LS2 9JT** (tel 0532-333969) quoting reference numbe 83/102. Applications (2 copies) stating age, giving details of qualifications and experience, naming three referees and providing a statement on how, if successful, the applicant would envisage fulfulling the requirements of the post should reach the Registrar not later than 14 September 1990. Applicants from overseas may apply in the first instance by telex (556473 UNILDS G) or facsimile (0532-336017 or 0532-334123), naming three referees, preferably at least one in the United Kingdom.

The University of Leeds is an equal opportunities employer

(4001)A

UNIVERSITY OF CAMBRIDGE
SCHOOL OF CLINICAL MEDICINE

Cytogeneticist/Research Assistant

Salary £11,088-£12,381

To join a molecular biology group investigating the role of chromosome 21 genes to the genetic disorders of Down syndrome and Alzheimer's disease. The work will entail mapping genes derived from embryonic brain cDNA libraries to metaphases of human chromosome 21 and mouse chromosome 16.

The Research group is actively searching for the genes involved in Alzheimer's disease and Atrioventricular Septal Heart Defect. Our gene cloning work has progressed to isolating a number of candidate genes which are being assessed anatomically by 'in situ' hybridisation and immunocytochemical analysis. We are looking for a cytogeneticist/research assistant with expertise in metaphase 'in situ' hybridisation to further our range of analysis and who will enjoy working within this integrative research approach to clinical disorders.

Applications should be forwarded to **Dr. Sarah-Jane Richards, Department of Medicine, Level 5, Addenbrooke's Hospital, Cambridge CB2 2QQ.**

The University follows an equal opportunities policy. (4131)A

INSTITUTE OF PSYCHIATRY
Department of Neuroscience

MOLECULAR BIOLOGY OF ALZHEIMER'S DISEASE
POSTDOCTORAL or GRADUATE RESEARCH ASSISTANT

A vacancy exists for either a postdoctoral fellow or graduate assistant (registration of PhD will be expected), depending upon the suitability of applicants, for a project on abnormal phosphorylation of microtubule associated protein tau. The work involves protein isolation, characterization, monoclonal antibody production and DNA cloning. The appointee will work with a technician on the same grant and in a multidisciplinary group studying neurodegenerative disease. The project is funded by The Wellcome Trust for 3 years and as such postdoctoral appointees will be eligible for a Wellcome Trust salary enhancement. (Salary range 1B £12,225-14,148; 1A £14,148-18,432 inclusive of London allowance. Prospective candidates are invited to contact Professor B H Anderton (071-703-5411 ext 3259).

Applications in the form of a CV including the names and addresses of two referees should be made to the **Personnel Office, Institute of Psychiatry, De Crispigny Park, Denmark Hill, London SE5 8AF** (Tel 071 703 5411 ext 3164) from whom further particulars may be obtained. (4149)A

Postdoctoral Position to study Varicella Latency and Gene Expression

Immediate opening for an individual with strong training in molecular virology-genetics to collaborate with a team studying varicella virus latency and gene expression.

Send curriculum vitae and names of three references to:

Dr. Donald H. Gilden, Professor and Chairman of Neurology
University of Colorado School of Medicine
4200 E. Ninth Avenue Box B-182
Denver, CO 80262

AA/EOE (NW5528)A

UNIVERSITY OF NEWCASTLE UPON TYNE
DEPARTMENT OF BIOLOGY

POSTDOCTORAL POSITION IN NEUROBIOLOGY

Applications are invited for a postdoctoral research position in Neurobiology, funded through the SERC Invertebrate Neuroscience Initiative. The project concerns the physiology, pharmacology and development of synapses which are made between identified neurones associated with the simple eyes of the locust. The successful applicant will join an active team which is involved in research into the mechanisms by which sensory information is processed within the insect optic lobes and ocellar pathways.

The position is tenable for two years, commencing between October 1990 and March 1991. Salary will be up to £14,703 per annum on the Grade 1A salary scale (£10,458-£16,665) according to qualifications and experience.

Enquiries and applications, which should include a curriculum vitae and the names and addresses of two referees, should be sent to **Dr. P. J. Simmons, Department of Biology, Ridley Building, University of Newcastle upon Tyne, NE1 7RU** (Tel: 091 222 6457, Fax. 091 261 1182) to arrive not later than 10th August 1990. (4146)A

SENIOR DIRECTOR

RESEARCH AND COLLECTIONS

This preeminet cultural and educational institution seeks a scientific visionary to exploit and influence its collection-based research and in so doing, raise its profile to that of a world leader. Under a new corporate mandate, the organization is pursuing a level of excellence in science that will guide the way into the next century.

As the Senior Director for Research and Collections, you will provide the leadership to forge comprehensive research programmes which will serve to understand the evolution, diversity and interrelationships of plants and animals, and the composition and evolution of the earth. Reporting to the institution's Director/Chief Executive, you will also be responsible for the development and preservation of collections of animals, plants, rocks and minerals for biological and geological research. You will explore the frontiers of knowledge where society's needs are greatest, and disseminate intelligence to science, government and the general public with the goal of enhancing the balanced relationship between man and nature.

You are a high-profile professional who brings to the position a doctorate, preferably in biology or geology, several years experience in leading-edge scientific research and a broad familiarity on subject of natural history. A seasoned leader, you are are bilingual have superior interpersonal abilities and well-developed communications skills.

If you possess the vision and drive to meet this unique challenge, please reply by 30 July, giving full personal details, quoting File No. E90603. Location: Ottawa.

RENAUD**FOSTER**
MANAGEMENT CONSULTANTS (NW5526)A

Suite 200, 440 Launer West, Ottawa, Ontario, Canada K1R 6XR Tel: (613) 594-3994 Fax: (613) 782-2228

The University of Glasgow
Department of Veterinary Pathology
Research Assistants in Molecular Virology

The following positions in molecular virology are available for 3 years from July 1990. Applications are invited from recent graduates with good degrees in molecular biology, virology or a related discipline. The appointments will be on the 1B Scale starting at £11,399 p.a.

(1) Development of Recombinant Vaccines to Equine Herpesviruses

Two research assistants are required to assist a group of 3 postdoctoral scientists developing vaccines to equine herpesvirus through deletion of genes affecting virulence. This group has been actively involved in the molecular biology of Equine Herpesviruses for several years and the work is now directed at producing a commercial vaccine. The successful candidates will be trained in the full range of molecular biology techniques.

(2) Characterisation of A New Retrovirus from Fish

A research assistant is required to characterise a new retrovirus isolated from fish. This project is part of a collaborative study with the Institute of Aquaculture at Stirling University, aimed at investigating the cause of a new epidemic disease of farmed fish in S.E. Asia. The Department of Veterinary Pathology has a major interest in the molecular biology and pathogenesis of retrovirus associated diseases. The successful candidate will be responsible for sequencing the genome of the new retrovirus isolate. Previous experience of techniques in molecular biology would be an advantage. Candidates, for this position, who possess a First Class Honours Degree will be permitted to register for a PhD.

Applications enclosing a full C.V. and the names of 2 referees should be sent to **Prof. David Onions, University of Glasgow Department of Veterinary Pathology, Bearsden Road, Bearsden, G61 1QH.** (4117)A

POSTDOCTORAL POSITION

Applications are invited for a postdoctoral position (2 years) in a research group studying genetics and regulation of virulence-associated properties in bacteria (*e.g. E coli* pili-adhesins, enterotoxins; for recent publications see: *Mol. Microbiol.* **3**, (1989) 1557. *Nature* **344**, (1990) 682).

PhD in biological sciences and experience in molecular genetics and biochemistry desirable.

Applicants should send curriculum vitae, statement of research interest, and a list of names and addresses of at least three references to:

Dr Bernt Eric Uhlin,
Department of Microbiology,
University of Umeå,
S-901 87 Umeå, Sweden (W7528)A

UNIVERSITY OF CAMBRIDGE
Assistant Director of Research in the Department of Physics

Assistant Director of Research in the field of Radio Astronomy to take up appointment from 1 October 1990, or as soon as possible thereafter. The successful candidate will engage in a strong programme of research in radio astronomy with the emphasis on experimental technique and will undertake teaching at graduate and undergraduate levels. The appointment will be for up to five years with the possibility of reappointment. The pensionable scale of stipends for an Assistant Director of Research, not ordinarily resident in College, is £14,169 a year, rising by nine annual increments to £20,469 (under review).

Further particulars may be obtained from the **Secretary of the Appointments Committee for the Faculty of Physics and Chemistry, Institute of Astronomy, Madingley Road, Cambridge CB3 0HA,** to whom applications (10 copies) including a curriculm vitae, the names, and addresses and telephone numbers of three referees and a full list of publications should be sent so as to reach him not later than 31 August 1990.

The University follows an equal opportunities policy (4150)A

WEST LAMBETH HEALTH AUTHORITY

St Thomas' Hospital

DEPARTMENT OF CLINICAL IMMUNOLOGY

MLSO 2

Salary Scale: £13,738-£17,579 inc.

Applications are invited from experienced laboratory workers for the above appointment in the Department of Clinical Immunology. This is a permanent appointment, and candidates will need to possess proven bench skills and good communication skills.

A successful postholder who could fulfil the responsibilities for training and development of laboratory staff should receive the award of two discretionary points. Opportunity is available for an appropriately qualified person to study for a higher academic qualification such as the Intercollegiate MSc in Medical Immunology or the MPhil degree by thesis. Attendance at management courses would be encouraged.

Ref: M/53.

MLSO I

Salary Scale: £10,464-£13,738 inc.

We are looking for a suitably qualified person to work in the Department of Clinical Immunology. This is a fully tenured and very interesting MLSO I*** appointment which carries the award of three discretionary points and we require someone who is well-motivated and enthusiastic.

The successful candidate, in addition to being involved in the routine laboratory investigations of the Department, will be expected to participate in training and in the evaluation of test procedures and reagents. The Department provides excellent facilities to gain further immunological experience and opportunities to obtain higher professional or academic qualifications such as the FIMLS or the MSc in Medical Immunology of London University.

Ref: M/54.

THE HOSPITAL IS WITHIN EASY WALKING DISTANCE OF WATERLOO AND WESTMINSTER STATIONS. WE OFFER AN INTEREST FREE SEASON TICKET LOAN SCHEME, SUBSIDISED MEALS AND SOCIAL CLUB.

FOR APPLICATION FORMS AND JOB DESCRIPTIONS please write to the Personnel Department, St Thomas' Hospital, London SE1 7EH, or telephone our 24 hour (Monday to Friday) answering service on 071-261 1185 quoting appropriate job title and reference.

Working towards equal opportunities. (4155)A

UNITED MEDICAL AND DENTAL SCHOOLS OF GUY'S AND ST THOMAS'S HOSPITALS

RESEARCH ASSISTANT

required in the Division of Anatomy at our Guy's Campus to work with Dr Caroline Wigley and Dr Jim Cohen on a project specifically concerned with cellular and molecular aspects of neuron survival and axon regeneration using tissue culture model systems and molecular techniques. This position is funded by the International Spinal Research Trust for three years in the first instance, starting from October.

Graduates in biological sciences with post-graduate experience in cell culture are welcome to apply. Further information contact Dr. Wigley (071-955 4410) or Dr. Cohen (071-955 4420). Salary on Research 1B scale £12,225-£13,503 inclusive depending on qualifications and experience.

Applications with full CV and the names and addresses of two referees to the **Personnel Officer, Staffing Office, UMDS, St Thomas's Campus, Lambeth Palace Road, London SE1 7EH** quoting reference G/ANA/577 by 27 July 1990. (4118)A

University of Waikato

Te Whare Wānanga o Waikato

Hamilton, New Zealand

Applications are invited for the following appointments:

LECTURER IN CHEMISTRY (Ref. A90/55)

Applicants should have a PhD or equivalent and preferably post-doctoral experience. Applicants are welcomed from specialists in any branch of the subject, but preference will be given to candidates with research interests in one or more of the following areas: Inorganic Chemistry; Analytical Chemistry; Industrial/Applied Chemistry. Information concerning the facilities available in the Chemistry Department and other enquiries of an academic nature should be addressed to the Chairperson, Associate Professor D W Smith (tel. (64) 71 562889 during business hours or fax (64) 71 560115).

UGC POSTDOCTORAL FELLOWSHIIP IN HONEY IDENTIFICATION (Ref. A90/33)

The Department of Chemistry and Biological Sciences invites applications for a University Grants Committee Postdoctoral Fellowship in Honey Identification. The appiontee would be expected to work on the development of a method of identification of the floral source of honeys by means of GC-MS analysis of trace organics. This would supplement the problematical method of pollen analysis currently used by the honey industry. The position will be available for a period of up to two years. Enquiries of an academic nature may be made to Dr A L Wilkins or Dr P C Molan (tel. (064) 71 562889; fax (064) 71 560115).

LECTURER IN PLANT MOLECULAR BIOLOGY (Ref. A90/57)

Applicants should have interests and experience in one or more of the following: developmental genetics of plants, molecular genetics of crop plants, primary and secondary plant metabolism, molecular basis of interactions between plants and other organisms. The Department of Biological Science has close links with agricultural research organisations and many opportunities exist to orient research towards the genetic improvement or basic metabolism of agricultural or horticultural crop species. The successful applicant is expected to teach in areas of genetics, biochemistry and botany according to his/her expertise. Enquiries of an academic nature may be made to Professor W B Silvester (tel. (64) 71 562889 during office hours or fax (64) 71 560115).

Current salary ranges per annum: Lecturer NZ$36,000-NZ$47,200; Postdoctoral Fellow NZ$36,000. Information on the conditions of appointment and method of application are available from the Academic Staff Registrar, University of Waikato, Private Bag 3105, Hamilton, New Zealand. (Tel (064) 71 562889, Fax (064) 71 560135). Applications quoting the appropriate reference number close **10 August 1990.**

Places for appointees children may be available in the creche run by the Campus Creche Society (Inc). The University welcomes applications from suitable people regardless of race, creed, marital status or disability. (W7531)A

Faculty of Agricultural and Environmental Sciences

Department of Plant Science

Macdonald College of McGill University

The Plant Science Department invites applications for a tenure track position, at the assistant professor level, in Sustainable Crop Production. Applicants must have expertise in an area of crop ecology. The preferred candidate should have a Ph.D. and postdoctoral experience in agricultural or related sciences with an interdisciplinary background. The successful candidate will be expected to teach at the undergraduate and graduate levels and develop a strong research program. Applicants should send a curriculum vitae and have three letters of reference sent to **Dr. Donald L. Smith, Plant Science Department, Macdonald College of McGill University, 21111 Lakeshore Road, Ste. Anne de Bellevue, Quebec, Canada, H9X 1CO** before November 15, 1990. (NW5530)A

POSTDOCTORAL RESEARCH ASSOCIATE

Brookhaven National Laboratory has a position available in the Medical Dept. for a recent Ph.D. trained in genetics and/or molecular biology. Experience in cytogenetic and molecular biology techniques, including DNA cloning and library construction, PCR, restriction and Southern analyses and in situ hybridization is required. Experience with mammalian/human tissue culture techniques is desirable. The work will include research on the molecular mechanism involved in the induction of cytogenetic effects by mutagenic/clastogenic agents, with emphasis on roles of replicative DNA polymerases and associated proteins. Interested candidates should contact Dr. M.A. Bender, Medical Department, Brookhaven National Laboratory, Associated Universities, Inc., Upton, L.I., NY 11973. *EOE M/F.* (NW5520)A

UNIVERSITY OF
Miami
SCHOOL OF MEDICINE

FACULTY POSITION IN MOLECULAR PHARMACOLOGY

he Department of Molecular and Cellular Pharmacology at the University of Miami School of Medicine is seeking applications for a tenure-track faculty position at the ASSISTANT OR ASSOCIATE PROFESSOR level. The position is available now at a rank and salary commensurate with experience. Generous lab space and start-up funds are available. Candidates must have a Ph.D. and/or M.D. degree, and an established record of research excellence involving molecular approaches to problems of pharmacological interest. The new faculty member will complement existing research efforts in the department, which include regulation of cardiac, smooth and skeletal muscle contraction, receptors and transmembrane signalling mechanisms, function and development of the nervous system, and regulation of gene expression.

Applicants should send a curriculum vitae, statement of past and future research interests, and the names and addresses of three references to **Dr. Lincoln T. Potter, Search Committee Chairman, Department of Molecular and Cellular Pharmacology, University of Miami School of Medicine, P.O. Box 016189, Miami, FL 33101.**

An Equal Opportunity/Affirmative Action Employer. (NW5518)A

DEOXYOLIGONUCLEOTIDE ANALOG DEVELOPMENT AND EVALUATION

Amgen has emerged as an innovative leader in the research and development of human therapeutics through applied biotechnology. As a result of our continued growth, we have opportunities available in Deoxyoligonucleotide Analog Development and Evaluation in our DNA Therapeutics Group, located in Boulder, Colorado, for the following positions:

RESEARCH SCIENTIST AND POSTDOCTORAL RESEARCH POSITIONS

The responsibilities of these positions include developing and executing new strategies for the characterization of the biological effects of oligodeoxynucleotide analogs in model systems.

Qualified candidates must have a Ph.D. in Biochemistry, Cell Biology, Virology or a related field. Supervisory experience is advantageous but not required.

Amgen offers an excellent compensation and benefits package and the unique opportunity to contribute to significant research programs on the forefront of biotechnology. If you feel that you have the necessary skills, qualifications and motivation and would like to be part of a company that places a high priority on its human resources, send a resume, in confidence, to:

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Department 2351
2045 32nd Street
Boulder, CO 80301

Equal
Opportunity
Employer (NW5332)A

AMGEN

UNIVERSITY OF GLASGOW
Department of Zoology
TEMPORARY LECTURER

Applications are invited for the above post which is available for three years from October 1990. The position is funded by the Wellcome Trust under a Research Leave Fellowship grant. Applicants should have experience in biochemistry, immunology or related subjects and a research interest in parasitology. The successful applicant will be expected to carry out some teaching and administration for the Department of Zoology and research facilities will be available either within the Department of Zoology itself or in the Zoology Section of the Wellcome Laboratories for Experimental Parasitology, which is sited at the Veterinary School campus. Applicants are particularly sought to teach the biochemistry and immunology of parasite infections or vector biology, but this is negotiable. Starting salary will be from £12,792 to £14,744, depending on age and experience, with appropriate annual increments thereafter.

Applications including a curriculum vitae and the names, addresses and telephone numbers of at least two adademic referees should be sent to **Professor R.S. Phillips, Department of Zoology, University of Glasgow G12 8QQ** to whom informal enquiries can be made on 041-339 8855 Extn 4776. (4139)A

UNIVERSITY OF CAMBRIDGE
University Lecturer in the Department of Chemistry

University Lecturer in Inorganic Chemistry to take up appointment on1 October 1990, The person appointed will be expected to teach and conduct research in the borderline areas between main-group and transition-metal chemistry. The appointment will be for three years, with the possibility of reappointment to the retiring age.

The pensionable scale of stipends for an University Lecturer, not ordinarily resident in College, is £14,169 a year, rising by eleven annual increments to £21,852. There is no grade of Senior Lecturer.

Applications (ten copies), including a curriculum vitae, an outline of research intentions and names and addresses of three referees should reach **Dr. M. F. Ingham Secretary of the Appointments Committee for the Faculty of Physics and Chemistry, Institute of Astronomy, Madingley Road, Cambridge CB3 0HA,** before 10 August 1990. Further details are available

The University follows an equal opportunities policy (4148)A

BUNDESGESUNDHEITSAMT

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Im **Institut für Arzneimittel Abt. Pharmazeut. Chemie, Biologie und Technolgie** ist die Stelle

des Leiters/der Leiterin

– Kzf. 329/90

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- Koordinierung und Organisation der Arbeit des Fachgebietes.
- Vertretung der Entscheidungen des Institutes zur Qualität gentechn. hergestellter Arzneimittel in den Arbeitsgruppen der EG.
- Mitarbeit an der Entwicklung neuer Richtlinien.

Anforderungen:
- Hochschulstudium der Biologie, Biochemie, Pharmazie oder Medizin; Promotion.
- vertiefte theoret. Kennatnisse und prakt. Erfahrungen auf den Gebieten der Molekularbiologie, Proteinanalyatik, Zellbiologie und Virologie.
- gute engl. Sprachkenntnisse.
- Organisationstalent und administrative Erfahrungen.

Angebot:
Bis Verg.-Gr. I a BAT; bei Erfüllung der Voraussetzungen ist die Übernahme in das Beamtenverhältnis bis A 15 BBesO möglich.

Nähere Auskünfte erteilt Herr Dr. Fuchs, Tel: (030) 4502 - 372. Schwerbehinderte Bewerber/innen werden bei gleicher Eignung bevorzugt berücksichtigt.

Bewerbungen mit aussagekräftigen Unterlagen erbeten unter o.g. Kzf. innerhalb von 10 Tagen an:
Bundesgesundheitsamt – Personalreferat – Postfach 33 00 13, 1000 Berlin 33. (W7537)A

MOLECULAR PARASITOLOGIST
ASSISTANT/ASSOCIATE PROFESSOR
SEARCH REOPENED

The Department of Zoology at the University of Georgia invites applications for a tenure-track position. Investigators working at the molecular/cellular level in any area of parasitology are encouraged to apply. Special consideration will be given to those with an expertise in areas such as recombinant DNA techniques and molecular separations. Departmental duties will include contributing to current teaching in parasitology to undergraduate students and developing a course in molecular parastiology at the graduate level. The successful candidate will have a prominent role in the Department of Zoology's current NIH training program in the molecular cell biology of parasites and vectors, and will be expected to develop a strong research program complementary to current departmental research which includes immunology, molecular biology, cell biology, biochemistry and ultrastructure of parasites. Current faculty are engaged in collaborative research in Brazil, Peru and Chile.

Send curriculum vitae; a statement of career objectives and current and planned research; and the names, addresses, and phone numbers of three or more references to:

Dr. Moises Agosin,
Chair, Search Committee,
Department of Zoology,
University of Georgia,
Athens, GA 30605,
Phone: 404-542-3439.

The University of Georgia

Applications received by September 15, 1990 are assured of consideration.

The University of Georgia is an Equal Opportunity/Affirmative Action Employer. (NW5515)A

ST GEORGE'S HOSPITAL MEDICAL SCHOOL
(University of London)
MOLECULAR BIOLOGY OF TRANSLATIONAL CONTROL
DIVISION OF BIOCHEMISTRY

Two Posts are available, for a Postdoctoral Scientist and a Grade 4 Research Technician, to carry out a project in Dr M J Clemens' Laboratory, funded by The Wellcome Trust. The work will involve cloning and characterization of mRNAs with extensive secondary structure which may be important in the translational control of eurkariotic protein synthesis. Both posts are available from 1 October 1990 and will be for up to 3 years. Applicants should preferably have some experience in DNA cloning and/or sequencing techniques.

Postdoctoral salary up to £16,470 including London Allowance, Research Technician salary up to £10,171 including London Allowance, depending on age and experience (both scales currently under review).

To apply please send curriculum vitae and names of two referees to **Dr M J Clemens, Division of Biochemistry, St George's Hospital Medical School, Cranmer Terrace, London SW17 0RE**, from whom further information can be obtained (telephone 081 672 9944 ext 55762). Closing date 10 August 1990. Please quote reference 81/90. (4127)A

ROYAL FREE HOSPITAL SCHOOL OF MEDICINE
(University of London)
DEPARTMENT OF PROTEIN & MOLECULAR BIOLOGY

Molecular Mechanism of Nucleoside Transport

An enthusiastic **Postdoctoral Researcher** is sought to join a team investigating the mechanism of nucleoside transport across mammalian cell membranes, using both protein chemical and cloning strategies in newly purpose-built laboratories. The project involves collaboration with groups at the Universities of Cambridge and of Edmonton in Alberta, Canada. Experience in molecular biology, protein chemistry or membrane biology desirable. For further information, please contact **Dr. S. Baldwin on 071-794 0500 ext. 4201.**

This MRC funded position is available for 1 year with salary on the Research 1A Scale plus £1,767 London Allowance.

Full job description available from the **School Office, RFHSM, Rowland Hill Street, London NW3 2PF** (071-794 0500 ext. 4262) to which applications by curriculum vitae (two copies) plus names and addresses of two referees, should be sent by 26 July 1990. *Please quote ref: PMB/RF/118.* (4156)A

Federal Research Centre of Agriculture
Institute for Small Animal Research Celle, F.R. German

MOLECULAR BIOLOGIST/MOLECULAR GENETICIS

For the establishment of a Research Unit of Molecular Genetics
tenured position is available. The applicant should initiate a resear
program aimed at chickens in one or combinations of the followi
areas:

- Structure of Genes and Regulation of Gene-Function
- Disease Resistance (Immunogenetics)
- Developmental Genetics

Qualifications: PhD or equivalent in Molecular Biology/Molecul
Genetics or a related field. Postdoctoral experience. Knowledge ir
wide spectrum of Molecular techniques is desirable. German and/
English language is essential.

The Institute pursues application oriented research on poultry a
other small farm animals.

Salary follows the public pay scheme (BAT IIa/Ib). If the applica
qualifies for the status of a German civil servant the position
A13/14.

The deadline for applications is: **August 15, 1990**, send to:

Federal Research Centre for Agriculture
Institut for Small Animal Research
Postfach 280,
3100 Celle, F.R. Germany (W7551)A

UNIVERSITY OF BRISTOL
DEPARTMENT OF PHYSIOLOGY

POST DOCTORAL RESEARCH ASSISTAN

Applications are invited from candidates with an interest in t
field of molecular neurobiology. A position funded by t
S.E.R.C. Invertebrate Neurosciences Initiative from 1st Septe
ber 1990 is available for three years to study how muscle ce
regulate the density and class of their membrane ion channe
Candidates should have (or expect to have) a Ph.D. degree.

Salary on RA1A scale (up to £16,665 per annum, salary sc
under review from 1.5.90).

Further information can be obtained informally from Dr. R.
Meach on (0272) 303472.

Applicants should send a curriculum vitae and the names a
addresses of two referees to the **Personnel Office, Senate Hou**
Bristol BS8 1TH. *Please quote reference 310.* (4152)A

UNIVERSITY OF EDINBURGH

Department of Molecular Biology

A POSTDOCTORAL POSITION IN MOLECULAR PARASITOLOGY

Applications are invited to join a group studying an important antigen of the malaria parasite, Plasmodium falciparum. The purpose of the project is to study the biological function of a protein being considered for a vaccine against malaria. Candidates with experience in protein and nucleic acid biochemistry will be preferred. The appointment will be for two years in the first instance. Salary on the 1A scale (£11,399-£18,165), with placement according to age and experience.

Applications by letter, together with curriculum vitae and the names of two referees should be submitted by the end of August 1990 to

Professor John Scaife,
Department of Molecular Biology,
University of Edinburgh,
Mayfield Road,
Edinburgh EH9 3JR.

PLEASE QUOTE REFERENCE NO 5786. (4130)A

UNIVERSITY OF NOTTINGH
Department of Physiology
Environmental Science

PLANT MOLECULAR BIOLO
RESEARCH FELLOW

The University is seeking to appo
Research Fellow in Plant Mole
Biology to work with Professor
Grierson in association with
AFRC Research Group in Plant
Regulation at the School of Ag
ture. Applicants should have a
and several years research e
ience. The appointment will be
October 1st 1990, or as soo
possible thereafter, for 3 years i
first instance at an appropriate
on the scale £11,399-£16,755.
person appointed will be expe
to make a significant contributi
the research programme in
Molecular Biology and to undert
limited amount of teaching
research supervision.

Applicants should send a *curric*
vitae and the names of two refere
Mr J D Linley, Personnel Office,
versity Park, Nottingham from w
further particulars may be obta
by the closing date of August 31.
phone: Nottingham (0602) 48
extn. 3355 REF. NO. 1354. (412

CHEMIST - INORGANIC/ PHYSICAL

AUS. $32,471 - $39,724 p.a.

If you are innovative, excited by new ideas, capable of solving complex problems, not afraid of making mistakes and know that you can rouse and generate interest in those working with you, then:

WE HAVE AN IDEAL OPPORTUNITY FOR YOU

This Division requires your innovative skills to enable it to contend with a range of problems and projects in timber preservatives and preservation processes and technology.

You will not only find ample scope for your creative capabilities, but you will become a vital member of an active research group. Your colleagues will be enthusiastic, similar-minded, friendly people at one of the Melbourne sites of our 300-strong, Australia-wide Divisions.

This position will require you to manage and work with a team to formulate, analyse and assess wood preservative chemicals, including complex metal systems and emulsion compositions. Close liaison and collaboration with other researchers in various wood products laboratories and especially with industry personnel, will enable you to put to use your strong interpersonal and communicative skills.

QUALIFICATIONS: PhD (or equivalent) in inorganic or metal complex chemistry is essential together with a first degree or equivalent in chemistry.

TENURE: Indefinite with Australian Government Superannuation Benefits available.

FURTHER INFORMATION: May be obtained by contacting Dr Harry Greaves on (613) 556 2218 or Facsimile (613) 543 6613.

APPLICATIONS: Stating relevant personal particulars, including details of qualifications and experience and the names of at least two professional referees are to be forwarded to:

The Chief,
CSIRO Division of Forestry &
Forest Products,
Private Bag No. 10,
Clayton, Victoria, 3168, Australia,
by 10th August, 1990.

(W7549)A

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AUSTRALIA

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CT 90/5/

BIOGEOCHEMIST

Biogeochemist (Research Assistant III) to collaborate in multi-disciplinary research program. Investigations of food webs in the NW Atlantic. MS (VG Micromass 903) analyses of stable isotopic enrichments in marine organisms with a focus on the trophic relationships of harp seals. Ph.D. in appropriate discipline preferred for this independent research position which is targeted to research investigations of trophic dynamics and population energetics.

Send vitae, most significant reprints and names and addresses of three references who can assess the candidate's demonstrated ability and potential for significant research achievement. Application deadline — 30 July 1990.

Send applications to:

Dr. Deane Renouf or Dr. William Montevecchi
Ocean Sciences Centre
Memorial University of Newfoundland
St. John's, NF
(FAX — 709-737-4000)

(NW5527)A

Maynooth College, Ireland

Postdoctoral Research Scientist

Applications are invited for a two-year appointment to a collaborative project funded by the EC. The project involves a study of cold activity and pathogenicity of bacteria symbiotic with insect parasitic nematodes. The position commences in September 1990. Experience in bacterial physiology and molecular biology is desirable. Initial salary of IR£17,000. Applications with curriculum vitae and names of three referees to **Dr Barbara Dowds, Dept. of Biology, St. Patrick's College, Maynooth, Co. Kildare, Ireland.** Tel: 353-01-6285222 ext 237 or 480. Fax: 353-1-6289063. Closing date for receipt of applications: 10th August, 1990.

(4162)A

UNIVERSITY OF ALASKA FAIRBANKS

SCHOOL OF FISHERIES AND OCEAN SCIENCES

Vacancy Announcement — External Vacancy
Faculty Tenure Track Position

TITLE AND RANGE:	Distinguished Professor of Fisheries Science School of Fisheries and Ocean Sciences, University of Alaska Fairbanks
SALARY:	$2100.80 Biweekly/DOE
JOB SUMMARY:	The University of Alaska Fairbanks is seeking applicants and nominations for the position of Distinguished Professor of Fisheries Science in the School of Fisheries and Ocean Sciences. The School is a newly-created unit, designed to integrate and enhance the fisheries and oceans programs within the University of Alaska. The position offers the opportunity to participate in its development and to provide intellectual leadership in this dynamic venture. As is usual for such senior positions, the successful applicant will be free to carve out his/her areas of emphasis from the numerous opportunities and challenges offered by Alaskan fisheries. He/she will assume a leadership role in fisheries research, teaching and public service for the new School, but will have considerable flexibility with respect to the specific activities. Fisheries is a dynamic area of science, and the School will be expanding its activities in a number of areas. There will be an opportunity to spend time at a number of off-campus sites in coastal areas of Alaska, and ultimately the permanent residence could be one of these locations. Initially, we plan to base the position at the Fairbanks campus.
SCHOOL SUMMARY:	The School of Fisheries and Ocean Sciences embraces the Institute of marine Science (which operates the Research Vessel *Alpha Helix*, the Seward Marine Center and the Kasitsna Bay Laboratory), the Juneau Center for Fisheries and Ocean Sciences at Auke Bay in southeast Alaska, the Fishery Industrial Technology Center in Kodiak, the Alaska Sea Grant College Program and the Marine Advisory Program. There are about 50 faculty, and an annual budget ranging from $15,000,000-$20,000,000.
QUALIFICATIONS:	The successful candidate will bring to the position a strong background in fisheries science and will have a distinguished record in university research and/or teaching, with a strong publication record appropriate to a leader in his/her field.
JOB DURATION:	Full time – Permanent.
APPLICATION:	APPLICANTS SHOULD SEND A STATEMENT OF INTEREST, A CURRICULUM VITAE, AND PROVIDE THE NAMES OF THREE REFEREES TO **CHAIRMAN, SEARCH COMMITTEE, OFFICE OF THE DEAN, SCHOOL OF FISHERIES AND OCEAN SCIENCES, UNIVERSITY OF ALASKA FAIRBANKS, ALASKA 99775-1080, USA. Telephone (907) 474-7531; FAX (907) 474-7204.**
OPENING DATE:	20 June 1990.
CLOSING DATE:	16 August 1990.

THE UNIVERSITY OF ALASKA IS AN EEO/AA EMPLOYER AND EDUCATIONAL INSTITUTION.

Persons hired by the University of Alaska must comply with provisions of the 1986 Immigration Control Act and are expected to possess a valid social security number.

Your application for employment may be subject to public disclosure should you be selected as a finalist.

(NW5523)A

Schering

Postdoctoral Fellow

If spearheading developments in allergy and immunology is what you have in mind, come to Schering-Plough Research.

Aggressive, goal-driven research gives Schering-Plough its competitive edge in the healthcare industry. But we need your progressive thinking to help effect significant breakthroughs in pharmaceutical research.

You will study the *in vivo* role of selected cytokines in immunologically-mediated disease. Exploring the mechanism of cytokine involvement involves employing molecular biological tools of PCR technology, *in situ* hybridization, and cellular immunology concurrent with the administration of anticytokine antibodies and cytokines by standard and novel means. Solid background in cellular immunology desirable. Additional skills in molecular biology would be a plus.

This position rewards talent with one of the highest stipends in the industry...unparalleled challenges... and the recognition for your ideas. For immediate response, please forward curriculum vitae to: Mr. G. Czuba, Schering-Plough Research, 60 Orange Street, Bloomfield, NJ 07003. We are an equal opportunity employer.

What you have in mind, we put in action.

Schering-Plough

(NW5512)A

PRIZES

POTAMKIN PRIZE FOR ALZHEIMER'S DISEASE RESEARCH

A prize of $100,000 will be awarded by th American Academy of Neurology to a per son in recognition of major contribution to the understanding of the causes and th prevention, treatment, and ultimately th cure for Alzheimer's disease and relate disorders.

Candidates may be nominated on a world wide basis from any of the biological dis ciplines including biochemistry, mol ecular biology, molecular genetics, phar macology, immunology, physiology, ce biology or neuropathology.

A nomination application, nominatin letter citing the scientific accomplish ments of the candidate in detail, two sup porting nominating letters, a curriculun vitae, and up to six (6) selected reprints i nine (9) complete sets are required.

The deadline for receipt of materials i November 1, 1990. No nomination will b considered unless it is complete. *Th awardee must be be present for the AA Scientific Program, at its annual meetin April 23-25, 1991 in Boston, Massa chusetts, U.S.A.*

Request application and submit nomina tion to:

Potamkin Prize for Alzheimer's Diseas
Research Award Committee,
American Academy of Neurology,
2221 University Avenue S.E. — Suite 335
Minneapolis, MN 55414
(612) 623-8115

The Potamkin Prize is funded through th philanthropy of the Potamkin Foundation

(NW5513)U

JOHN INNES INSTITUTE

A research position is available from November 1990 to study the molecular genetics of floral homeotic genes in Antirrhinum majus. The research will involve isolating genes by transposon-tagging and analysing their mode of action using a range of molecular and genetic techniques. Applicants should ideally have experience in genetics, molecular biology or a related discipline. This post is in the laboratory of Dr Enrico Coen and Rosemary Carpenter.

This period appointment will be to the Higher Scientific Officer grade: Salary scale £10,678 to £14,909 per annum (Pay award pending). Non-contributory superannuation. Candidates should have a first or upper 2nd class honours degree with at least 3 years relevant post qualifying research experience. Equal opportunities employer.

Applications in writing with the name of two referees should be sent to the **Personnel Officer, John Innes Institute, John Innes Centre for Plant Science Research, Colney Lane, Norwich, NR4 7UH, UK,** quoting ref: GEN/494 no later than 9.8.90. (4160)A

MEDICAL RESEARCH COUNCIL
MRC MAMMALIAN DEVELOPMENT UNIT

Applications are invited for a three year

MRC STUDENTSHIP

starting in October 1990 and leading to a PhD degree of the University of London and a

SCIENTIFIC OFFICER

to start immediately. The research would focus on regulation of gene expression in mammalian development. Applicants for either position should have, or expect to obtain, a good honours degree in a biological subject, preferably including some molecular biology and genetics.

Curriculum vitae with your daytime telephone number and names and addresses of two referees, should be sent not later than Friday 27 July to: **The Secretary, MRC Mammalian Development Unit, Wolfson House, 4 Stephenson Way, London NW1 2HE** (telephone 01 387 9521). (4146)A

MRC
Medical Research Council

UNIVERSITY OF SUSSEX — School of Biological Sciences

Post-doctoral Research Fellow

(1) A post-doctoral research fellow is required to join a group studying th structure and function of viral oncogenes and the role of these oncogenes i immortalising human primary cells. The project will involve site-directed mut genesis, DNA sequencing, construction of novel oncogene bearing vectors, an PCR. Candidates with experience of molecular biology techniques and prote chemistry are encouraged to apply, however, suitable candidates from oth backgrounds will be considered as training in these techniques will be provide The successful candidate will join a well funded group with good technic support. The appointment will initially be for three years with a likely extensio of two years.

(2) We are seeking a post-doctoral research fellow to join a group analysing th defective gene in the human disorder Cockayne's syndrome. The successf applicant will be involved in the isolation of this gene and the study of i product. In particular, we are interested in the involvement of this gene produ in the development and maintenance of the nervous system. Ideally, the su cessful candidate will have some experience of molecular biology technique However, training in PCR, DNA sequencing, in situ hybridisation and DN mapping will be provided. This project is funded by the Wellcome Trust.

Salaries will be on the Research 1A scale £10,458-£16,665 (under review Applicants should submit a full CV and the names of two referees to:

Mrs. R. Warton, E.A.P.S. III
University of Sussex
Falmer, Brighton BN1 9QT

(4165)A

UNIVERSITY OF OXFORD

RESEARCH ASSISTANT

Required for 3 years for a biochemical study of the effects o aldehydes on the lens and on lens proteins. These studies ar relevant to cataract, and putative anti-cataract drugs will also b investigated. Starting salary up to £11,975 depending on age an experience. Further information may be obtained from **Dr J. Harding, Nuffield Laboratory of Ophthalmology, Walton St Oxford** to whom applications should be sent with full CV and th names and addresses of two referees. (4166)A

THE UNIVERSITY OF ADELAIDE

SOUTH AUSTRALIA

tes applications from both women and men for a

TENURABLE LECTURESHIP

vailable immediately in the

DEPARTMENT OF MICRIOBIOLOGY AND IMMUNOLOGY

Department teaches Science Medical undergraduate and tgraduate students and has an ellent research record in study- infectious agents and the dis- es they cause. Current research as include the pathogenesis of d immunological responses uced by a number of enteric terial, protozoan and persis- t virus infections. The Depart- nt has particular strength in lecular biology, monoclonal tibody technology, immuno- emistry and cellular immuno- y. Opportunities also exist for laboration with relevant re- rch groups in the adjacent stitute of Medical and Veterin- Science and Royal Adelaide spital. Applicants should hold a .D. degree or equivalent with propriate teaching ability and perience and should have a sig- icant track record in research thin the field of Immunology. uties of the appointee will clude lecturing, primarily to dergraduate Science students, pervision of research work of postgraduates, and sole responsibility for some of the teaching programmes and administrative tasks of the Department. Preference will be given to applicants providing evidence of ability to develop active research interests in areas which would add to or complement existing research interests of the Department. Further information may be obtained from Professor Chris Burrell, Head of Department of Microbiology and Immunology. Annual Salary: A$33,163 × 7 – A$43,096 plus para-clinical loading of A$8,049. It is University policy to encourage women to apply for consideraion for appointment to tenurable academic appointments. Holders of full-time tenured or tenurable academic appointments have the opportunity to take leave without pay on a half-time basis for a specific period of up to ten years where this is necessary for the care of children.

Applications in duplicate quoting reference number 1725, giving personal particulars (including whether candidates hold Australian permanent residency status), resume names and addresses of three referees should reach the Director, Personnel Services, University of Adelaide, GPO Box 498, Adelaide, South Australia 5001, Australia, Telex UNIVAD AA 89141, Facsimile (08) 224 0464 not later than 16 August 1990. From 1 January 1991 Roseworthy Agricultural College will merge with the University of Adelaide. The University is an Equal Opportunity Employer. (W7552)A

HIV TRAINING OPPORTUNITY

Immediate openings for MD or PhD postdoctoral fellows interested in pathogenesis and immunology of HIV or HIV-associated opportunistic infections. This research program links the infectious disease unit at New England Medical Center with the basic sciences retrovirology and immunology programs at Tufts Medical School, and is supported by a newly founded NIH Training Grant. If interested, please contact **Dr. Gerald Keusch, Program Director,** at **617-956-7001** or **New England Medical Center, 750 Washington Street, Box 41, Boston, MA 02111.** *The medical center is an equal opportunity employer.* (NW5517)A

POSTDOCTORAL POSITION

In molecular or cellular neurobiology available Oct. 1990. Candidate with Ph.D. in biochemistry, molecular biology, cell biology, neuroscience, or related area sought to investigate the structure and function of recently discovered receptors for gangliosides in the brain. Techniques in neurochemistry, membrane biochemistry, cellular neurobiology and/or molecular biology will be applied to probe these novel neural recognition proteins.

Please send curriculum vitae and names of three references to: **Dr. Ronald L. Schnaar, Dept. of Pharmacology and Molecular Sciences, The Johns Hopkins School of Medicine, 725 N. Wolfe St., Baltimore, MD 21205.** (NW5529)A

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nature

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FACULTY POSITIONS IN MOLECULAR NEUROBIOLOGY

THE OHIO STATE UNIVERSITY

The Biotechnology Center, in collaboration with academic departments within the Colleges of Biological Sciences, Medicine, and Pharmacy, invites applications for three joint tenure track faculty positions. Successful candidates will occupy new laboratories on the Neurobiology floor of the Biotechnology Center and join existing faculty whose research efforts focus on gene expression in nervous tissue during development and aging. This group will shortly expand to ten faculty. Applicants for **Assistant Professorships** should demonstrate exceptional potential to develop strong research programs that apply tools of molecular biology to address significant problems in neurobiology. Senior candidates with ongoing programs of excellence will be considered for an **Associate Professorship**. Qualified molecular neurobiologists working with vertebrate or invertebrate systems will receive careful consideration. Candidates who utilize transgenic mice in the course of their research are especially encouraged to apply. The Ohio State University is establishing an outstanding program in molecular neurobiology and neuroscience which is reflected by quality research facilities and highly competitive start-up funds and salary.

Screening of candidates will begin on August 15, 1990. Applicants should submit a curriculum vitae, statement of research plans, and names and addresses of three references to **Dr. Anthony Young, Chairperson, Molecular Neurobiology Search Committee, Ohio State Biotechnology Center, Rightmire Hall, 1060 Carmack Road, Columbus, Ohio, 43210.** *The Ohio State University is an Equal Opportunity/Affirmative Action Employer.* (NW5477)A

UNIVERSITY OF OXFORD

Department of Earth Sciences,
Parks Road, Oxford, OX1 3PR, U.K.

POSTDOCTORAL RESEARCH ASSISTANT 1A

Applications are invited from recent doctorates in isotope geology/igneous petrology to work on a NERC-funded project "Regional investigations of Andean magmatism by radiogenic-isotope geochemistry", involving mass-spectrometry, chemical analysis, and field work in South America. This post is available for up to three years from 1 January 1991 or ASAP thereafter. Applications with curriculum vitae and names of two referees, as well as requests for more details, to Dr. S. Moorbath, F.R.S. (0865-272035) by 30 September.

The University is an equal opportunity employer (4167)A

POST-DOCTORAL POSITION

The Department of Biochemistry and Cell Biology at RICE UNIVERSITY announces the availability of a post-doctoral position for biophysical and physiological studies of hemoglobins and myoglobins. Strong background in either rapid reaction techniques or molecular biology is desired. Projects will involve the construction, preparation, and characterization of site directed mutants. Available immediately. Salary will depend on experience. Send résumé and three letters of reference to **Dr. John S. Olson, Professor, Department of Biochemistry and Cell Biology, Rice University, P.O. Box 1892, Houston, Texas 77251.** *Rice is an Equal Opportunity/Affirmative Action Employer.* (NW5531)A

STUDENTSHIPS

MEDICAL RESEARCH COUNCIL
Human Genetics Unit
Western General Hospital
Crewe Road, Edinburgh EH4 2XU
Tel: 031 332 2471

MRC Postgraduate Studentship to study the Molecular Genetics of Schizophrenia

The student will join a multidisciplinary team using reverse genetics to map and study genes associated with familial schizophrenia. For further details, suitably qualified molecular biology graduates from the U.K. or the E.E.C. should telephone Dr J Bard or Dr D St. Clair at the above Unit. (4164)F

SHELL RESEARCH
THORNTON RESEARCH CENTRE
and
DURHAM UNIVERSITY

CASE Studentship

Investigations of the molecular dynamics of fluids by infrared and Raman spectroscopy

The relationship between molecular structure, flexibility and intermolecular interactions at the microscopic scale on the one hand and the bulk viscometric properties of liquids on the other hand is of great topical interest. In this project infrared and Raman spectroscopy will be used to study the relationship between the industrially important bulk properties (e.g. viscosity, elasticity) of model lubricants, and the dynamics and interactions of their constituent molecules. The most up-to-date equipment is available for spectroscopic studies at high temperatures and pressures, and for computer-aided molecular modelling.

Applicants should have a good honours degree (or equivalent) in Chemistry, Physics or Chemical Physics. SERC standard conditions plus an additional £750 per annum to the student. There will be an opportunity to work at a modern industrial research laboratory. Applications to **Dr. F. D. Wayne (051-373-5665)** at **Shell Research, Thornton Research Centre, P.O. Box 1, Chester CH1 3SH**; or **Dr. J. Yarwood (091-374-3136), Chemistry Department, Durham University, South Road, Durham DH1 3LE** as soon as possible. (4153)F

TWO CASE RESEARCH STUDENTSHIPS

A BIOCHEMICAL AND MOLECULAR APPROACH TO ELUCIDATE MICROBIAL ADAPTION TO EXTREME ENVIRONMENTS.

Universities of Aberdeen & Nottingham and Unilever Research

Applications are invited for two AFRC Cooperative Award Studentships. These studentships present a valuable opportunity to enter into a collaborative research programme between two Universities, Industry and the AFRC. Industrial preservation techniques are designed to stress microorganisms to the point of minimal survival. Microorganisms, however, have a wealth of genetic and biochemical defence mechanisms to promote their survival under stress. Understanding these mechanisms is essential if more effective antimicrobial strategies are to be developed.

Interested students with relevant qualifications should contact **Dr I Booth, Department of Molecular and Cell Biology, University of Aberdeen, Marischal College, Aberdeen AB91AS (Tel: 0224 273152)** or **Dr G Stewart, Department of Applied Biochemistry and Food Science, University of Nottingham, Sutton Bonington, Loughborough, Leics LE12 5RD (Tel: 0602 484848 ext 8370).** (4123)F

UNIVERSITY OF READING
DEPARTMENT OF MICROBIOLOGY

PhD Studentships

Applications are invited from individuals who possess or expect to obtain a 1st or upper 2nd class degree in Microbiology, Molecular Biology or a related subject, for the following studentships:

1. An MRC studentship to work with Dr. D. J. Evans on the structure/function relationships of the envelope glycoproteins of HIV, involving gene cloning and expression studies using the poliovirus system developed in Reading (Nature 322, 81-82 and 339, 385-388). Tel. (0734) 318893.
2. A Reading University studentship to work with Dr. P. Poole on the regulation of dicarboxylate oxidation in Rhizobium, involving the purification and cloning of malate dehydrogenase from both free-living and symbiotic species. Tel. (0865) 275306.

The Department has excellent facilities for advanced research and a lively research atmosphere, and will shortly be moving to new purpose built buildings as part of the School of Animal and Microbial Sciences.

Informal telephone enquiries on the indicated numbers are welcomed. Applications, including a CV with the names of two referees, should be made to the supervisors at the following address: **Department of Microbiology, University of Reading, London Road, READING, RG1 5AQ. (0734) 318901.** (4136)F

UNIVERSITY OF KENT
AT CANTERBURY ■■■■

PHD OPPORTUNITIES IN THE BIOLOGICAL LABORATORY 1990

Biochemistry Biotechnology Microbiology
Cell and molecular biology

The following Ph D studentships are available for October 1990

SERC Biotechnology directorate earmarked studentships

Protein engineering studies on the following of lysozyme (Professor R B Freedman)

Characterization of a thermostable lipase (Professor R B Freedman and K A Stacey)

SERC CASE studentships*

Biotreatment of pesticide residues in effluents (Dr R G Burns/A H Marks Ltd)

The genetics of production of a secondary metabolite by a pseudomonad (Professor K A Stacey/ICI)

SERC Quota studentships

Cell and molecular biology of the neuronal cytoskeleton (Drs A J Baines and M J Carden)

Molecular biology of protein secretion in bacteria (Dr T Hirst)

* These studentships carry an extra stipend from collaborating companies.

Applicants should have or expect to obtain by October 1990 a first or upper second class degree in a relevant subject. Please supply as soon as possible enclosing a brief c.v. to individual project supervisors from whom further information can be obtained.

Biological Laboratory, The University of Kent Canterbury, Kent CT2 7NJ.
Tel: 0227 764000. Fax: 0227 763912. (4126)F

KING'S COLLEGE LONDON
University of London

Protozoan Predation in the Rhizosphere

The rhizosphere is the interface between the plant root and the bulk soil: through it pass most of the mineral nutrients required by plants for growth and up to 40% of the photosynthetically-fixed carbon which is lost from roots. The rhizosphere, which is a region of high microbial activity, is therefore of intrinsic interest as a specialised ecosystem and an understanding of the processes that take place within it is of practical value in terms of increasing plant productivity and disease resistance in an environmentally acceptable manner. Of particular interest is the role of predatory organisms in the rhizosphere as they have potential as biocontrol agents.

Applications are now being sought from suitably qualified graduates (IIA or better in a science-based subject) to study protozoan predation in the rhizosphere. The project will involve establishing experimental microcosms at King's College and glasshouse experimentation at the Institute of Horticultural Research, Littlehampton. The successful applicant will join a team of scientists studying several aspects of the rhizosphere, attend international meetings and visit collaborators in overseas laboratories. The studentship attracts a supplement of £1250 in excess of basic stipend.

Enquiries should be accompanied by a c.v. and the names of two referees and should be sent as soon as possible to: **Dr. M. Bazin, Division of Biosphere Science, King's College London, Campden Hill Road, London W11 7AH.** (4147)F

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and the Wellcome Foundation,
Beckenham, Kent

MRC COLLABORATIVE ESEARCH STUDENTSHIP: ONTROL OF β-OXIDATION BY HYPOGLYCAEMIC COMPOUNDS

plications are invited from tably qualified graduates for a D Studentship to work on the odification of β-oxidation in lls and mitochondria by hypo- ycaemic compounds which e inhibitors of β-oxidation. The n is to determine the control engths of the individual steps β-oxidation with different con- ions, and to relate these to etabolism in the fasting, fed d diabetic states. The supervi- rs will be Dr H.S.A. Sherratt, partment of Pharmacological iences, and Dr L. Agius, partment of Medicine, Univer- y of Newcastle upon Tyne, and C.I. Pogson, Biochemical iences, Wellcome Foundation d., Beckenham, Kent.

e basic MRC Studentship ould be generously supple- ented by the Wellcome Found- ion and opportunities will be ailable to attend scientific eetings. Enquiries and applica- ns, including a c.v. and the mes of two referees, should sent as soon as possible to **Dr Agius, Department of Medi- ne, University of Newcastle on Tyne, Newcastle upon ne NE2 4HH, Tel: 091 2226000, tension 7033.** (4144)F

UNIVERSITY OF EDINBURGH
MEDICAL SCHOOL
Department of Biochemistry

2 Postdoctoral Research Fellowships

ION CHANNEL STRUCTURE AND MECHANISMS

Applications are invited from Post-doctoral (or near-Postdoctoral) scientists to work for 3 years in the laboratories of Richard Ashley and David Hulmes. Both positions are funded on the AR1A scale. One post is supported by the British Heart Foundation as part of a multi-disciplinary approach to investigate pore conformations of the cardiac calcium release channel. A second post (funded by the Wellcome Trust) is for work on a novel brain calcium channel involved in calcium signalling. We routinely use a number of different techniques and hope to attract candidates from a very wide range of backgrounds including electron microscopy of macromolecules, molecular biology and electrophysiology. Further written details about each post and about the Department of Biochemistry are available from the **Personnel Office, 63 South Bridge Edinburgh EH1 1LS,** with whom applications by letter, including c.v. and the names and addresses of two referees, should be lodged by 31st August 1990. PLEASE QUOTE REFERENCE NO. 5784. (4116)E

SEEDS OF TOMORROW THROUGH GENETIC ENGINEERING

Applications are invited for **junior or senior fellowships** available in the period October 1st, 1990 to September 30th, 1991 in the Training Network on Applications of Genetic Engineering and Molecular Biology for Seed Improvement.

This Network is hosted by the Commission of the European Communities and 50 laboratories in Plant Molecular Biology in the Member States.

Conditions: Ph.D. degree or equivalent.
The postgraduate research has to be carried out in a country different from the fellow's resident country.

For information on project opportunities and host laboratories write to:

Professor Diter von Wettstein
Department of Physiology
Carlsberg Laboratory
Gamle Carlsberg Vej 10
DK-2500 Copenhagen Valby
Denmark

(W7534)E

NUROFEN PAIN RELIEF PROJECT

TRAVEL FELLOWSHIPS

To endorse its support of current research in the field of pain mechanisms and management, the Nurofen Pain Relief Project is offering travel fellowships to UK health professionals and research scientists.

Worth up to £1,000, the fellowships are designed to help meet the travel costs, of those not yet in senior positions, invited to present new work at meetings and symposia.

For full information and an application form, send an SAE to the address below:

Nurofen Pain Relief Project (TF)
4/5 Cloisters House
8 Battersea Park Road
London SW8 4BG

NUROFEN

(4163)E

KING'S COLLEGE SCHOOL OF MEDICINE AND DENTISTRY
of King's College London
Department of Immunology

RESEARCH FELLOW IN IMMUNOLOGY OF HIV INFECTION

Applications are invited for a three year research fellowship to undertake a study prospectively evaluating immunological and clinical parameters as guides to the management of patients with human immunodeficiency virus (HIV) infection. The post is based in the Department of Immunology where the successful candidate will investigate various aspects of immune functioning in more than 150 HIV-positive patients. The post would suit someone keen to develop research experience and work towards an academic career. The successful applicant will be joining a stimulating group of collaborators and will be expected to participate in the research activities of the department.

Salary will be based upon the NHS Registrar scale or the equivalent non-clinical scale. For further details and informal discussions please contact Dr. M. Peakman on 071-326 3588. Applications in the form of a full curriculum vitae and the names and addresses of two referees should be sent to the **Secretary of the School, King's College School of Medicine and Dentistry, Bessemer Road, London SE5 9PJ** by 27th July 1990. (4115)E

UNIVERSITY OF OXFORD MERTON COLLEGE

Junior Research Fellowships 1991

The College proposes, if candidates of sufficient merit present themselves, to elect in 1990 to two or three Junior Research Fellowships. These Fellowships are open to both men and women. Candidates must not have accumulated more than six years in post graduate study or research by 1 October 1991.

Details of the awards and of the method of applying for them may be obtained by writing to the **Warden's Secretary, Merton College, Oxford, OX1 4JD.** Closing date for applications is Friday 5 October 1990. (4122)E

EMBO

European Molecular Biology Organization

LONG TERM FELLOWSHIPS IN MOLECULAR BIOLOGY

AUTUMN 1990 AWARDS

Next deadline: 15 August 1990

EMBO long term post-doctoral fellowships are awarded to promote the development of molecular biology and allied research in Europe and Israel. To be eligible a candidate must hold a doctorate degree and the exchange must involve a laboratory in Western Europe or Israel. EMBO fellowships are not, however, awarded for exchanges between laboratories within any one country. Long term fellowships are usually awarded initially for one year, but with the possibility of renewal for a second year. In cases of exceptional scientific merit, however, the selection committee can award a 2-year fellowship. The fellowship comprises a return travel allowance for the fellow and any dependents and a stipend and dependents' allowance.

Since the selection procedure may include an interview, candidates are requested to respect the deadline for complete applications which is 15 August 1990. Successful candidates will be notified of their awards immediately after the meeting of the selection committee, which is on 29 October 1990.

Application forms and further details may be obtained from **Dr. J. Tooze, Executive Secretary, European Molecular Biology Organization, Postfach 1022.40,69 Heidelberg 1, F.R. Germany.** Inquiries should be accompanied by a self-addressed adhesive label. (W7235)E

ASSISTANTSHIPS

UNIVERSITY OF NOTTINGHAM
DEPARTMENT OF ZOOLOGY

Postdoctoral Research Assistant

Applications are invited for a three year postdoctoral research assistantship funded by the MRC at the MRC Experimental Parasitology Research Group in the Department of Zoology. The project will involve the investigation of cytokine regulation during chronic infection with gastrointestinal parasitic nematodes and will focus on parasite factors responsible for interference in cytokine activity and downregulation of inflammatory and host-protective immune responses.

Candidates should possess a PhD or equivalent degree, should have an interest in parasitic infections and above all should be self-motivated and prepared to work in a multidisciplinary research group. Previous experience in cellular immunology, particularly in the measurement or evaluation of cytokine activity would be an advantage.

The salary will be in the 1A range £10,458-£11,736 pa (under review).

Closing date is 6 August 1990 and the appointee should be prepared to commence work soon afterwards. Enquiries and applications, including a comprehensive CV and the names of two referees, should be addressed to **Dr J M Behnke, Department of Zoology, University of Nottingham, Nottingham NG7 2RD.** (4143)P

DYSON PERRINS LABORATORY
DEPARTMENTAL ASSISTANTSHIP

The biosynthesis of ß-lactam antibiotics is the focus of a multidisciplinary approach at Oxford, closely related to the recently established Oxford Centre for Molecular Sciences. Applications are invited from highly motivated biological scientists for a Departmental Assistantship, to work as part of the team working in this and related biosynthetic areas. Experience in enzymology and/or molecular biology is necessary. The successful applicant will be expected to contribute to the supervision of the group, as well as undertaking research studies. Salary is on the RS1A scale £10,458-£14,169 (under review) according to age and experience and will be for 3 years.

Applications for this pensionable post, including c.v. and the names of two referees, should be sent to **Professor J.E. Baldwin, Waynflete Professor of Chemistry, Dyson Perrins Laboratory, South Parks Road, Oxford, OX1 3QY.** Further particulars may be obtained through Oxford (0865) 275671. *The University of Oxford is an equal opportunities employer.* (4114)P

ANNOUNCEMENTS

British-Italian Collaboration in Research and Higher Education

Call for proposals

The Italian Ministry of Universities and Research in Science and Technology (MURST) and the British Council invite proposals under a new bilateral programme to promote scientific co-operation between research and higher education institutes in the two countries.

It is envisaged that most proposals will be for joint research projects of up to 3 years' duration in science, engineering, agriculture, social applications of science or medicine, or for collaborative action on professional formation, course design/validation or similar aspects of higher education in those areas. The programme will provide grants for a series of exchange visits, and key criteria for acceptance will be mutual benefit and the intention to develop long-term links.

Attention is also drawn to existing programmes aimed specifically at exploratory visits.

Closing date: 30 September 1990.

Further information and Notes for Applicants from:
Ufficio Relazioni Internazionali, MURST, Lungotevere Thaon di Revel 76, 00196 ROMA
Tel: (06)-3969941 Fax (06)-392209 Telex 612548 MUR 1
or Dr. Penny Aspden, Science Officer, The British Council, Via IV Fontane 20, 00184 ROMA
Tel: (06)-4826641 Fax (06)-4814296
Telex 622231 BRICON 1.
(4135)G

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AGROECOLOGY AND CONSERVATION ISSUES IN TEMPERATE AND TROPICAL REGIONS

International Symposium

University of Padova, Italy, Department of Biology

September 26-29 1990

Topics of the Conference

- Animal and plant diversity and conservation
- Food web systems and energy flow patterns
- Plant and animal interactions
- Sustainable agriculture and farming conversion
- Agroforestry
- New trends in biological control practices
- Recovery and conservation of soil vitality
- Landscape ecology in agriculture
- Economy of agriculture ecosystems

A focus of the symposium will be to emphasize the interrelation and transfer of research and application in sustainability and environmental problems between temperate and tropical areas of the world.

290 people are attending from 42 countries. A limited number of posters is still accepted. Attendance is open: contact the Secretary by fax.

Organizing Committee

F. Golley, Athens, GE, UCA; G.G. Lorenzoni, Padova, Italy; O. Ferro, Padova, Italy; M.G. Paoletti, Padova, Italy; B.R. Stinner, Wooster OH, USA; L. Toniolo, Padova, Italy; A. Zamorani, Padova, Italy; A. El Titi, Stuttgart, West Germany; H. Chun-Ru Beijing, China; J. Bellot, Zaragoza, Spain; L. Ryskowski, Poznan, Poland.

Secretary

M.G. Paoletti, Padova, Italy, G.G. Lorenzoni, Padova, Italy. Department of Biology, Padova University, via Trieste, 75 35100-Padova Italy. Tel: 049-8286309-08, 049-8286182, fax: 049-8072213-8286300, telex 430176. (W7527)M

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INTERNATIONAL WEEKLY JOURNAL OF SCIENCE

MODERN MANAGEMENT OF HEADACHE

5th/6th November 1990, London

As the Cinderella of the Neurosciences, headache has had little attention paid to it and hardly any research was done on the subject. Three important advances have activated new interest: the new classification of headache; the long held theories of the pathogenesis of migraine have been challenged; and there are now new treatments based on specifically targeted receptors. This intensive course, given by specialists from the Charing Cross and National Hospitals will give the background to these advances, discuss the different types of headache and how new treatments are assessed. There will be patient demonstrations giving delegates a unique opportunity to hear about their symptoms and treatment.

This course will interest those seeking an update or rapid understanding of the modern management of headache, particularly clinicians, R&D personnel and those in marketing, product management and sales with responsibility for headache and migraine drugs.

Details from:

Fiona Morgan, IBC Technical Services Ltd.
Bath House, 56 Holborn Viaduct, London EC1A 2EX.
Tel. 0935 77964 (Int. +44 935 77964)
Fax. 0935 78148 (Int. +44 935 78148) (4161)A

BIOTECH USA

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NOVEMBER 27–29, 1990
WASHINGTON D.C.

CALL FOR PAPERS AND POSTERS
on the following topics

Pharm/Biotech:

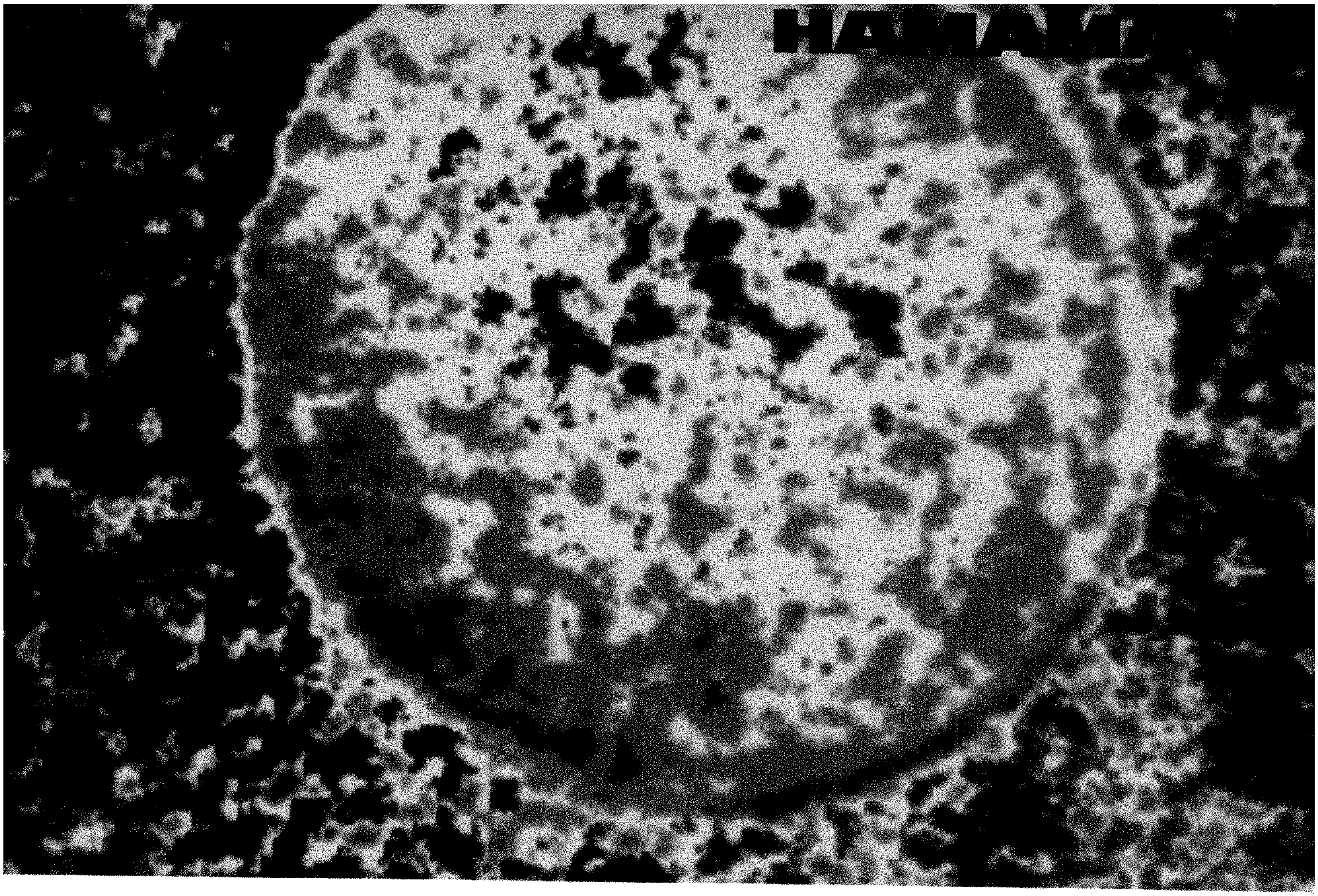
- Producing Pharmaceuticals in Plants and Animals
- Rational Drug Design
- Fermentors for Biopharmaceutical Production
- Protein Purification Techniques
- Diagnostics and Devices

Ag/Biotech:

- Engineering Plants for Pest and Disease Resistance
- Agronomic Improvement of Livestock
- Food Engineering

Please send abstracts by September 1 to:

Gina Amatruda
CMC/BIOTECH USA
200 Connecticut Ave.
Norwalk, CT 06856–4900 (NW5516)C



This fluorescence polarity image of a fertilized sea urchin egg obtained with a Hamamatsu ultra-high sensitivity camera shows that the fluidity of the egg's plasma membrane decreases after fertilization. Photonics allows the study of these phenomena without destruction.

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325–6, Sunayama-cho, Hamamatsu City, 430 Japan
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THE THIRD INTERNATIONAL CONFERENCE
PEACE THROUGH MIND/BRAIN SCIENCE

August 5th – 10th 1990
Hamamatsu, Japan

Aims of the Conference:

The brain is an organ which, in human beings, governs high-order mental functions such as knowledge, emotion and will. Study of the brain is viewed as forming one of the greatest frontiers upon which science and technology can throw light.

Further elucidation of the brain and mental functions must have a high impact on the advancement of science and technology, and hence open up the way to new developments in science. Moreover, this impetus would play an important role in deepening our understanding of the nature of human-beings, as well as a key role in contributing to world peace.

The aim of this conference is to apply new opto-science and technology to research on the brain and mental functions and thereby further advance this technology in the international world of science.

Presentations

The number of presentations will amount to approximately 30. (Sessions 1–6, Mon-Wed)

Participating countries:

America: The Soviet Union: Sweden: The United Kingdom: Canada: France: Japan: Others.

Attendees

Estimated number to total 200. (50 Non-Japan: 150 Japan)*
*Attendance is by invitation only.

Language:

English

Executive Committee:

Chairman Teruo Hiruma, President Hamamatsu Photonics K.K.; Kanji Torizuka, President, Fukui Medical School; Ken-ichi Uemura, Professor, Hamamatsu University School of Medicine; Yukio Tateno, Director, Division of Clinical Research, National Institute of Radiological Sciences.

Program Committee:

Chairman Ken-ichi Uemura,	Professor, Hamamatsu University School of Medicine;
Britton Chance	Professor, Department of Biochemistry and Biophysics, School of Medicine, University of Pennsylvania;
Eiichi Tanaka,	Consultant, Hamamatsu Photonics K.K.;
Guy McKhann,	Director of the Mind/Brain Institute, The Johns Hopkins Medical Institutions, Johns Hopkins University;
Henry N. Wagner, Jr,	Director, Divisions of Nuclear Medicine and Radiation Health Sciences, The Johns Hopkins Medical Institutions, Johns Hopkins University;
Sydney Brenner	Medical Research Council, Molecular Genetics Unit;
Toshiro Yamasaki,	Section Head, National Institute of Radiological Sciences;
Yasuyoshi Watanabe,	Department Head, Osaka Bioscience Institute;
Yoshiharu Yonekura,	Lecturer, Kyoto University.

SCHEDULE/TIMETABLE

DATE	MORNING	AFTERNOON	EVENING
SUNDAY 5th AUGUST		REGISTRATION	REGISTRATION MIXTURE PARTY
MONDAY 6th AUGUST	OPENING SPECIAL LECTURES (Dr. Y. YONEKURA)	SESSION 1 BEHAVIOR AND BRAIN (1) Chairman: Dr. G. McKHANN	EVENING TALK "WHAT SHOULD WE DO FOR THE FUTURE" Co-ordinator: Dr. H. WAGNER
TUESDAY 7th AUGUST	SESSION 2 BEHAVIOR AND BRAIN (2) Chairman: Dr. H. WAGNER	SESSION 3 TIME RESOLVED SPECTROSCOPY AND IMAGING OF TISSUES Chairman: Dr. B. CHANCE	RECEPTION (18.30-20.30)
WEDNESDAY 8th AUGUST	SESSION 4 MOLECULAR ANALYSIS OF NERVE SYSTEM Chairman: Dr. S. BRENNER	SESSION 5 NEW TECHNOLOGY Chairman: Dr. E. TANAKA	EVENING TALK "WHAT SHOULD WE DO FOR THE FUTURE" Co-ordinator: Dr. H. WAGNER
THURSDAY 9th AUGUST	STATEMENT (OPTIONAL) PHOTONICS TOUR	SATELLITE SESSION IN JAPANESE	WINE PARTY (19.00-20.30)
FRIDAY 10th AUGUST	(OPTIONAL) Informal Meeting about above session		

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FAX: 0534+53+6673

Conference Office:

Conference Secretary, N. Takashita, Planning Division,
Hamamatsu Photonics K.K.
325-6, Sunayama-cho, Hamamatsu City, Japan
TEL 0534-52-2141 (Ex. 2730) FAX 0534-56-7889

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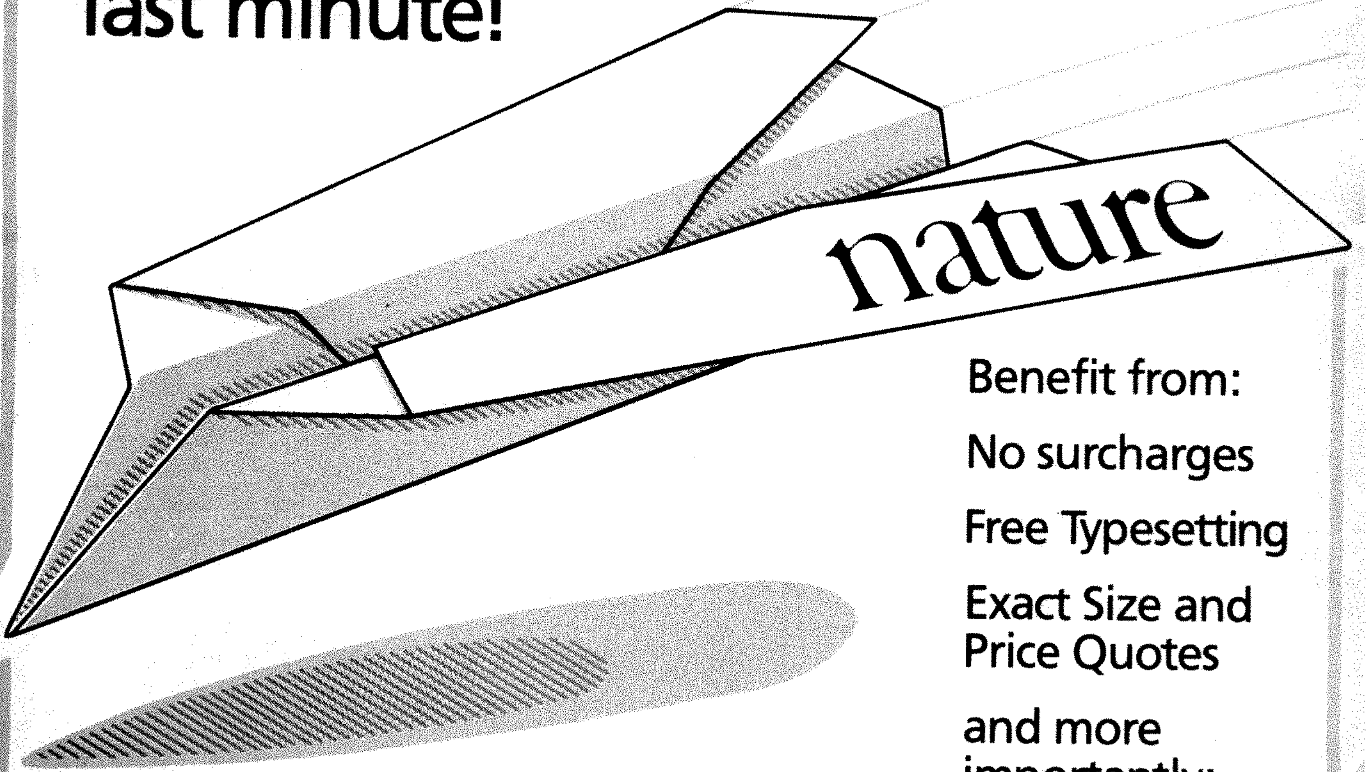

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International Science and Technology Courses

Advances in biotechnology: animal cell culture

16–27 September 1990, Canterbury.

Director: Dr. N. Jenkins, Lecturer in Biochemistry and Head of the Animal Cell Culture Unit, University of Kent.

Course fee: £985

★★★★

The Developmental and conservation of soil fertility

16–28 September 1990, Aberdeen.

Directors: Professor D. Atkinson, Professor of Land Resource, Professor J.W. Parsons, Professor of Soil Science and Oliver Brandon, Project Coordinator of the Aberdeen Centre for Land Use.

Course fee: £1080

★★★★

Environmental policy and management: an international forum.

7–14 November 1990, Alfriston, East Sussex.

Director: Brian D. Clark, Executive Director of the Centre for Environmental Management and Planning, Aberdeen University.

Course fee: £895

★★★★

Modern approaches to plant virology: virus transmission, detection and resistance.

17–29 March 1991, Dundee

Directors: Professor B.D. Harrison, CBE FRS, Head of Virology Department & Dr. A.T. Jones, Research Leader, Scottish Crop Research Institute.

Course fee: £1170

Recent advances in farming systems research: an international seminar.

2–12 April 1991, Edinburgh.

Director: Barry Dent, Professor of Rural Resource Management, Edinburgh School of Agriculture.

Course fee: £1260

★★★★

Wildlife economics and management: policy and practice.

2–21 April 1991, Canterbury/Jersey

Directors: Dr. I. Swingland, Director of the Durrell Institute of Conservation & Ecology, University of Kent & Dr. D. Waugh Training Officer of the Jersey Wild-Life Preservation Trust.

Course fee: £1760

★★★★

Advances in biotechnology (2): bioreactors in industry.

14–25 April 1991, Edinburgh.

Directors: Professor C.M. Brown, Director, International Centre for Brewing and Distilling. Dr. L.R. Weatherley, Dept. of Chemical & Process Engineering, Heriot-Watt University, Edinburgh.

Course fee: £1180

★★★★

Biosensors and their application to biotechnology, medicine and the environment.

11–21 May 1991, Newcastle-upon-Tyne.

Director: Professor A.K. Covington, Professor of Electroanalytical Chemistry, Faculty of Medicine, University of Newcastle-upon-Tyne.

Course fee: £1165

All course fees are inclusive of accommodation

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Further information and application forms are available from **Courses Department, The British Council, 10 Spring Gardens, London SW1A 2BN. Tel: 71 930 8466**or your local British Council representative.
For France, **Science Office, 11 rue de Constantine, 75007 Paris, Tel: 45 55 95 95.**

(W7522)C

120 YEARS AGO

The fabled alligator captured in the Thames some months ago has been surpassed by a hippopotamus disporting itself in the Seine. The scarcity of water has been so great in the Jardin des Plantes, that his majesty had been taken by his keepers to the river for his daily bath, securely held, as was thought, by his chain. One day, however, he snapped his chain during his gambols, to the no small dismay of the *blanchisseuses* and steam-boat passengers, one lot of whom he threatened to demolish at a mouthful. Several keepers who attempted to board him were treated to a playful ducking, but after the upsetting of a good number of small boats, he was at length captured and hauled ashore. The poor brute must have thought that the good old times of the pre-glacial epoch had returned.

Though the Russians boast, and with good reason, of the quality of their tea, a vast quantity of rubbish is sent to that country from China for consumption by the poorer classes. This is known as Brick Tea, and is frequently made up of the sweepings of the manufactories and warehouses mixed with bullock's blood and other refuse, and compressed into hard cakes or bricks; for use it has to be boiled. In some parts of India the natives use a similar kind of brick tea to make a thick kind of drink more like soup.

Tea contains an active principle called "theine" and a volatile oil, it also contains about fifteen per cent. of gluten or nutritive matter, and about twenty-five per cent. of tannin or astringent matter. The effect of theine upon the human system is to excite the brain to greater activity, but whether or not it soothes the vascular system by preventing the rapid waste of the body, is a point upon which physiologists are not quite agreed. Theine, however, if taken in excessive quantities produces tremblings, irritability, and wandering thoughts. It has been recommended that when these symptoms show themselves, cocoa should be used as a beverage for a few days.
"Tea", J. R. Jackson.
From *Nature* July 14, 1870.

100 YEARS AGO

Mr. L. W. Wiglesworth, writing from Brunswick, says:– "I am indebted to a friend for the following observation. A squirrel, in leaping from a height of 33 feet to the ground, caused itself, by means of curving its tail strongly to one side just before alighting, to swerve in its course and so avoid some hard substance upon which it would otherwise have fallen. It landed safely upon a more suitable spot. If no one has done so already, I should like to call attention to the use of the squirrel's tail as a steering and balancing organ during the animal's passages through the air."

For the first time since the establishment of the Gardens of the Zoological Society there is now to be seen there one of the ancient breed of the English wild cattle, Earl Ferrer having presented to the Society a fine young bull, which he captured in Chartley Park, Staffordshire. From Garner's "Natural History of Staffordshire" it appears that the wild ox formerly roamed over Needwood Forest. In the thirteenth century William de Farrarus caused the park of Chartley to be separated from the forest, and the turf of this extensive enclosure still remains almost in its primitive state. Here a herd of wild cattle has been preserved down to the present day, and they retain their wild characteristics, like those at Chillingham.
From *Nature* July 10, 1890.

50 YEARS AGO

While difficult to define, the sensation of 'stuffiness' is easily recognized subjectively by everyone. Since the sensation can be caused by overheating, it is essential in work on stuffiness to mak sure that the human subjects of the experiment are comfortable a regards warmth. Following the procedure of earlier investigation on human comfort, a numerical scale, the substantial linearity c which has been demonstrated statistically, was allotted to th various comments on sensations of stuffiness and freshness expressed by the subjects. The reactions of 'sessions' of about ten peopl each, tested separately, could then be averaged. By such methods has been shown that sensation of stuffiness increases, *ceteris paribu* with air temperature, according to a definite smooth curve.

An experimental gas fire has been built consisting of a blackene iron plate 14 in. × 16 in. with nine chromel alumel thermocouple embedded in it at strategic points. Its temperature can be varie over a wide range by control of the gas rate, and can be measured b means of the thermocouples. Spectrometric investigations hav shown that the fire approximates to a 'black' or 'grey' body, an confirmed the wave-lengths of maximum energy calculated fro temperature measurements.

The results so far obtained suggest that 'stuffiness' is associate with values of λ max. of about 3 μ, 4.1 μ and 4.8 μ, with regions o freshness in between; where a low temperature for 'neutral' freshness, that is, neither fresh nor stuffy, means a wave-length associated with stuffiness and vice versa. With the longer wave-lengths the quantity of radiation is smaller, the subject nearer to the fire and therefore less evenly warmed by it, and the results more erratic.
"Radiation and 'Stuffiness'", K. W. Yarnold and H. D. N. Fitzpatrick.

In his presidential address on "Wolf-Children" delivered before the Folk-Lore Society on February 21 (*Folk-Lore*, **51**, 9; 1940), Dr. J. H. Hutton deals with the cases recorded from classical antiquity down to the present time of children having been reared by animals, especially wolves. The earliest record of a wolf-child which he has discovered in northern Europe is that reported by William Dilich in 1341 as occurring in Hesse. Two Lithuanian cases of wolf-children were described by Connor in his "History of Poland", published in 1698, since when no cases have been described in Europe. Many recent cases, however, have been reported from India, mostly from the United Provinces, and also from Central India and Africa.

Wolves are not the only animals which have been said to have carried off and suckled children. In classical antiquity, Atalanta is stated to have been suckled by a bear and Cyrus by a bitch, while in recent times bear-children, jaguar- and leopard-children have been recorded in India, and lion-children and a baboon-child in Africa. It is a remarkable fact that no stories of the kind have been reported from the New World, where examples of wolf- and bear-children might have been expected from North America and jaguar- and puma-children from South America.
From *Nature* July 13, 1940.

10 YEARS AGO

Citing circumstantial evidence of the use of poison gas by Soviet troops in Afghanistan, the US Congress has taken the first step towards ending an eleven-year moratorium on the production of chemical weapons. By-passing the White House — which has nevertheless so far raised no objection — the House of Representatives last week approved the army's use of funds to renovate facilities at its Pine Bluff Arsenal in Arkansas to house the production of so-called binary chemical weapons.

Binary weapons, the latest thing in the military gas-man's catalogue, offer safe storage and use; two relatively innocuous chemical components which react to produce a military gas only after the weapon has been primed. The US Army has been working on their development since the mid-1960s.
"Chemical weapons: Congress agrees".
From *Nature* July 10, 1980.

IN NEXT WEEK'S ISSUE

Competing theories of cosmology assessed ■ Testis determining gene ■ On the energetics of running ■ Increased nitrate concentrations in Arctic snow ■ Scanning tunnelling microscopy images of DNA at atomic level ■ Ozone and hydrogen peroxide in forest air

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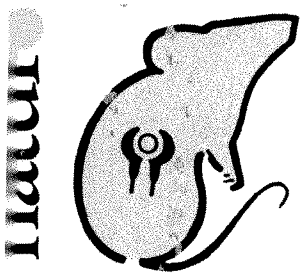

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. ANTIGEN PROCESSING ND PRESENTATION

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. VACCINES AND NTIBODIES

ésar Milstein (MRC Cambridge)
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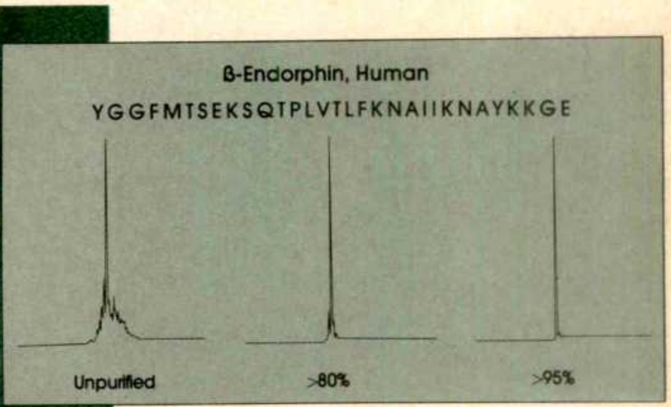

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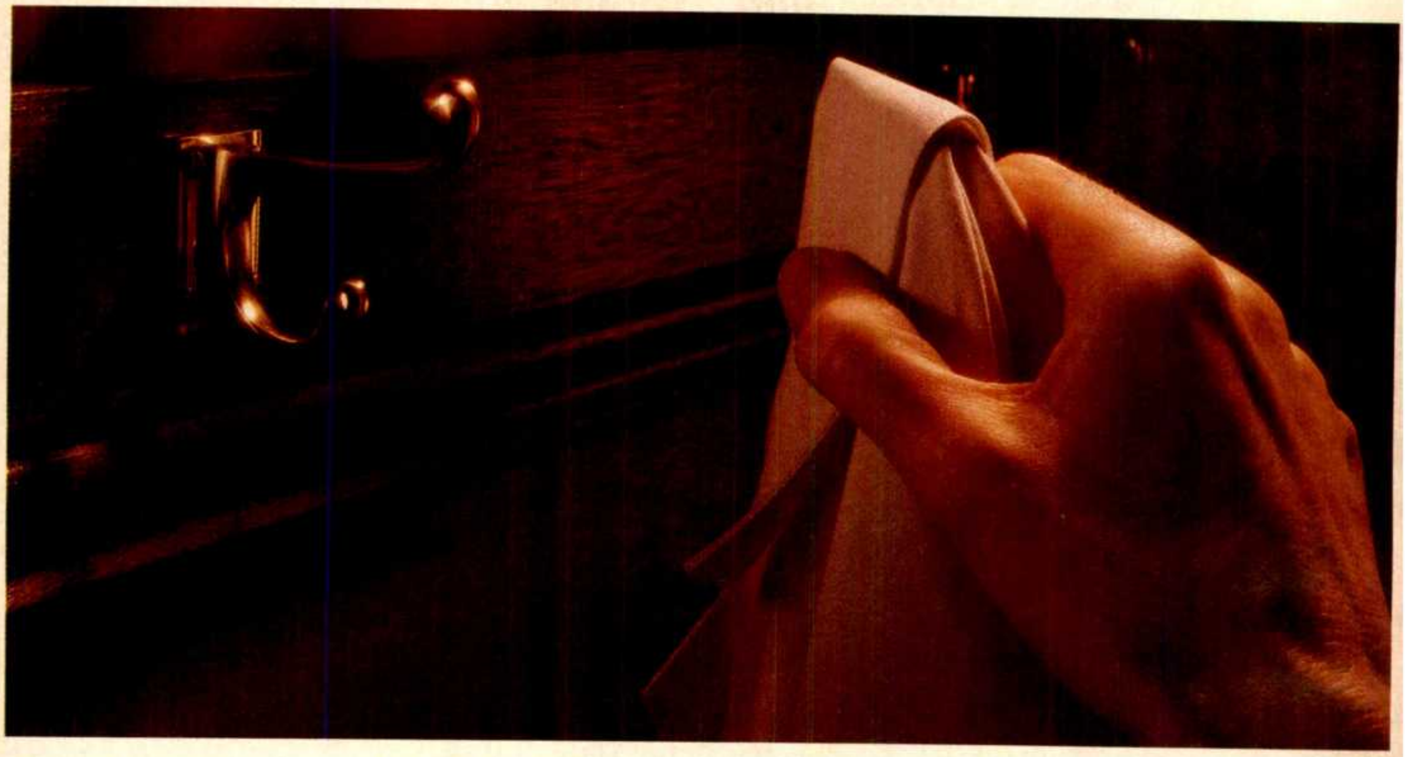

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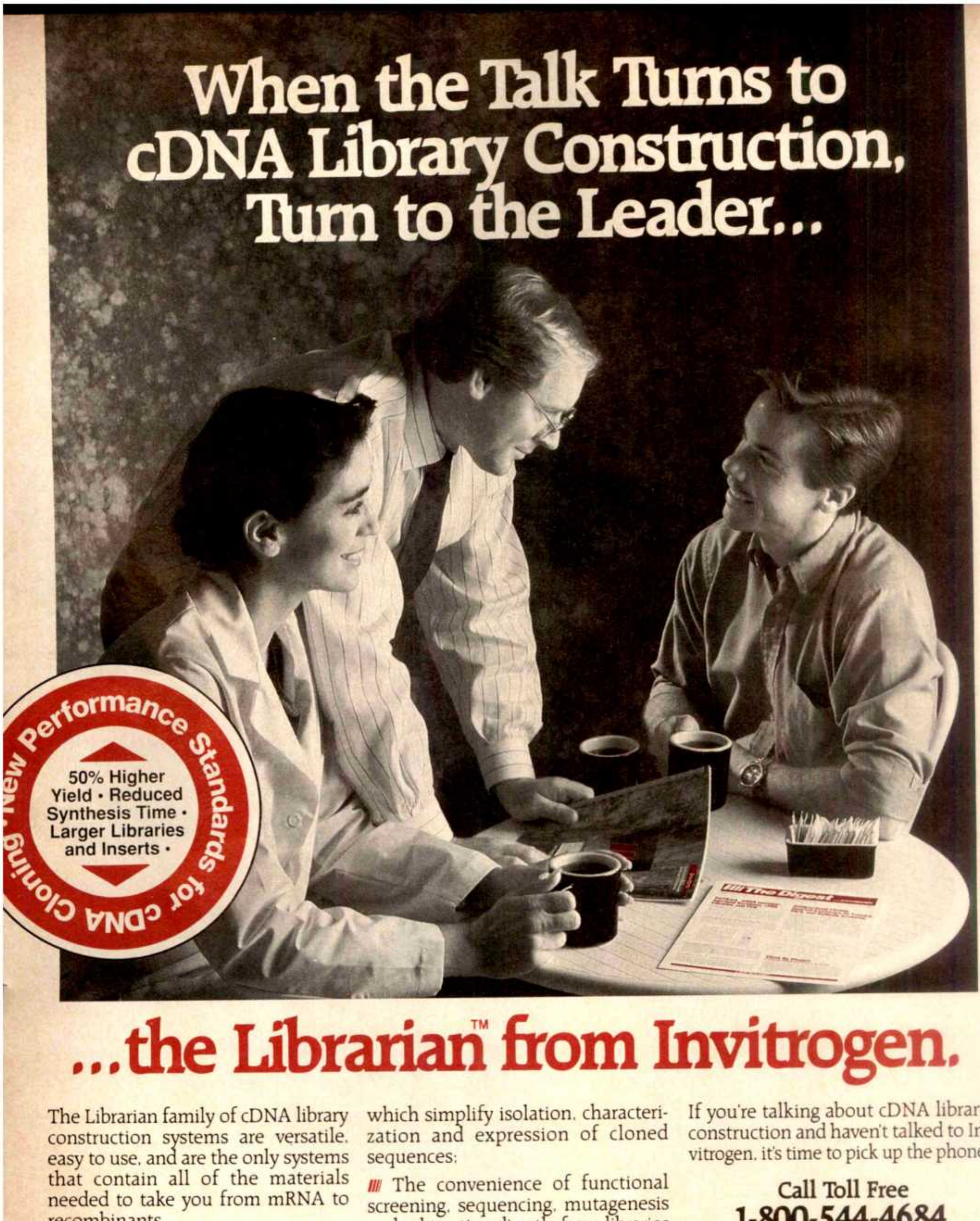
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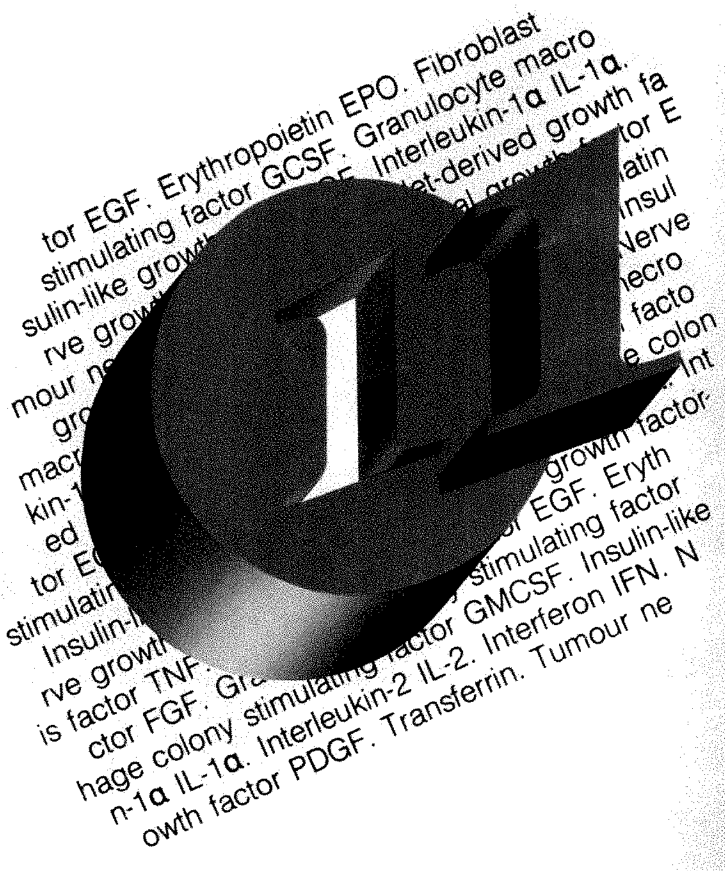

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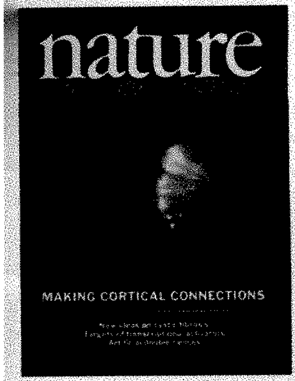


nature

26 July 1990

Vol. 346 Issue no. 6282

◀ The cover shows projection neurons in an organotypic culture with the fluorescent dye DiI from a cocultured target area of the visual cortex. Cortical projections established in culture are remarkably similar to those *in vivo*. See page 359.

THIS WEEK . . . THIS WEEK . . . THIS WEEK . . .

CF defect modelled

The product of the cystic fibrosis gene is thought to be a transmembrane transporter protein with an ATP-binding domain on its cytoplasmic face. Predictions of the secondary and tertiary structures of this domain reveal a similarity to that of adenylate kinase, but the predicted structure suggests that genetic defects in a hydrophobic loop, rather than in the ATP-binding site, cause cystic fibrosis, page 362. A number of new cystic fibrosis mutations include a cluster of substitutions at residues conserved among ATP-binding transport proteins, further strengthening the case that the gene product really is such a transport protein, page 366. See News and Views, page 312.

Gene activation

Cloning of the human transcriptional activation factor TFIID reveals a remarkable evolutionary conservation of the TFIID core, and a completely distinct N terminus with structural motifs analagous to those of other regulatory factors and co-factors, page 387. On page 390, Gasch *et al.* show that the plant *Arabidopsis thaliana* contains two distinct genes for the TFIID factor, the first direct evidence for the presence of multiple TATA binding factors in a eukaryote. Recent research on the mechanisms of eukaryotic transcriptional activators using cloned TFIID has led to apparently contradictory results. On page 329 Ptashne and Gann suggest how the conflicting conclusions may be reconciled.

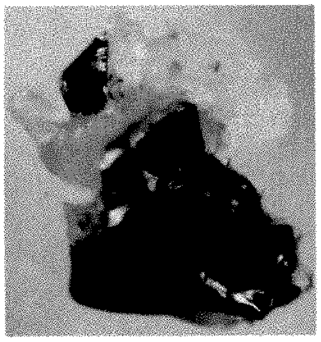
Chemistry imitates life

DNHs, a new type of artificial compound combining a double-helical structure with selective interaction features analogous to those of nucleic acid bases, may provide structural and functional models for the study and mimicry of the functions of DNA. Pages 339 and 314.

Guppy love

A long-term study of guppies living in environments with different predators bears out the prediction that reduced adult survival selects for earlier maturation and increased reproductive effort. Pages 357 and 313.

Uncommon carbide



Crystals of silicon carbide, one of the rarest minerals in nature, from a diamond-containing kimberlite. Hexagonal (deep blue) and cubic (milky-coloured) polymorphs can be seen. Actual height of specimen 0.7mm. See page 352.

Kidney disease

The onset of experimentally induced disease in the animal model of acute proliferative glomerulonephritis — an inflammatory disease of the kidneys — is causally related to increased production of transforming growth factor beta, suggesting a possible new therapy for the human disease, page 371.

Light programme

The synthesis of polymer gels that undergo a reversible, discontinuous volume phase transition on exposure to light (page 345) holds out the prospect of producing energy-conversion devices for the direct utilization of energy derived from sunlight. Potential applications might include artificial muscles, switches and memories.

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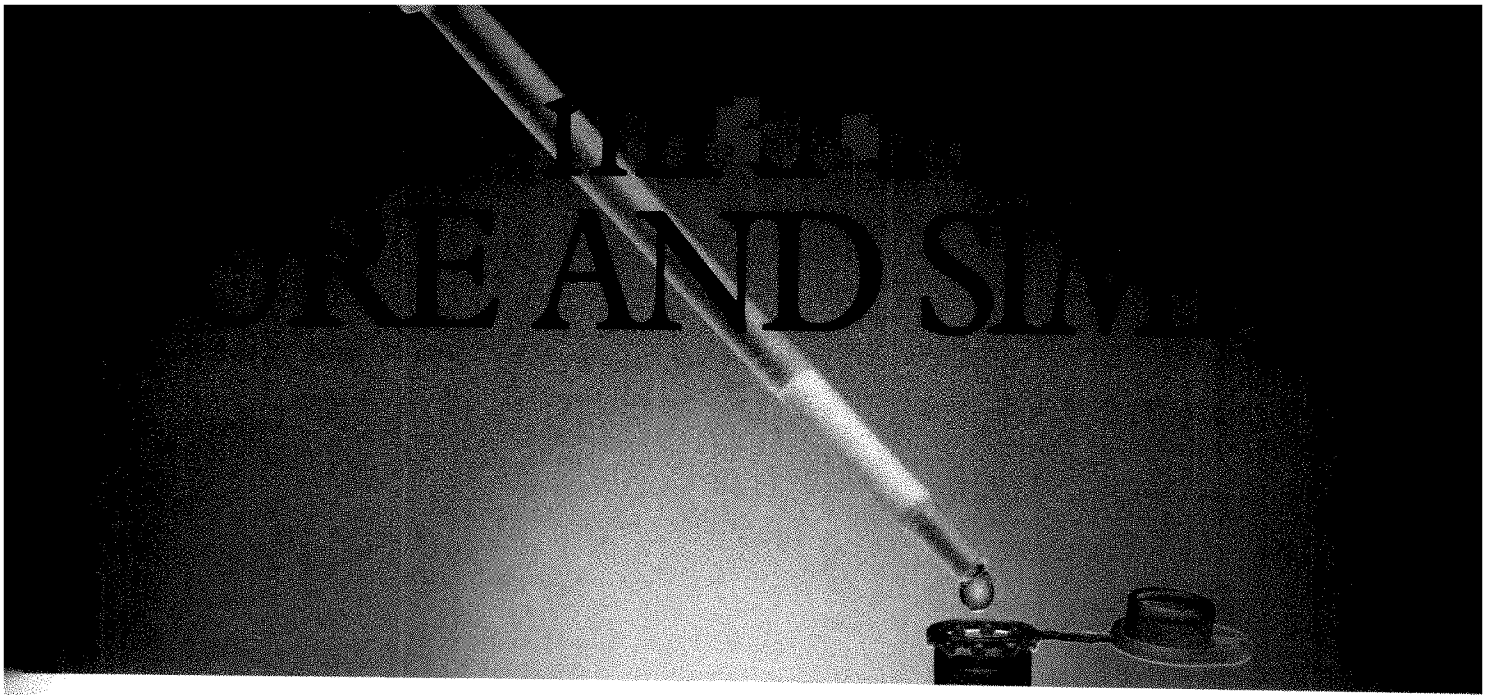

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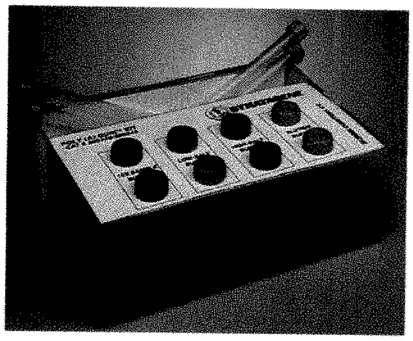

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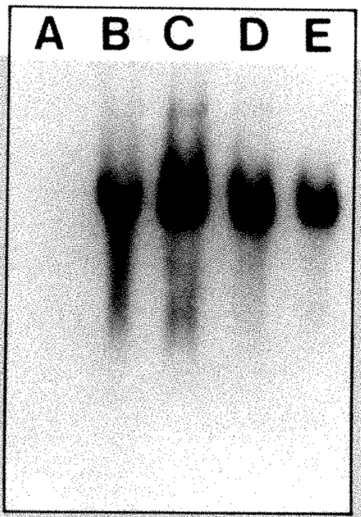

Total RNA was extracted from transgenic and non-transgenic mouse liver using the Stratagene RNA isolation kit. One mg of transgenic total RNA was passed twice over a Poly (A) Quik™ push column, and the poly A$^+$ fraction was purified. Samples were electrophoresed on a VAGE™ vertical formaldehyde agarose gel, vacuum blotted, UV cross linked, and hybridized to a DNA probe to detect the presence of the transgene.

A: 5 μg non-transgenic mouse liver total RNA.
B: 5 μg transgenic mouse liver total RNA.
C, D & E: 500, 175 and 75 ng of poly A$^+$ enriched RNA from the Poly (A) Quik™ push column.

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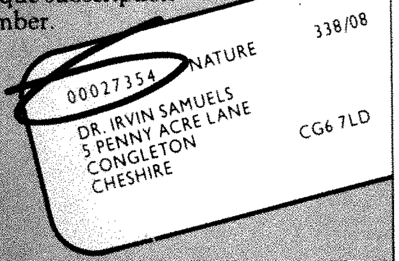

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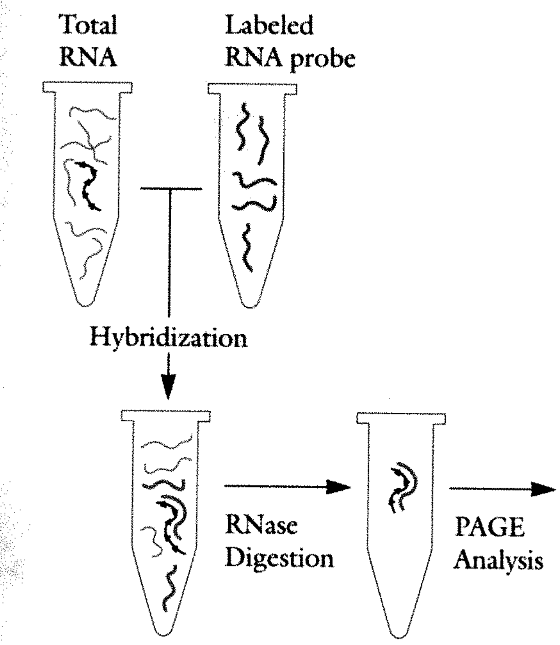

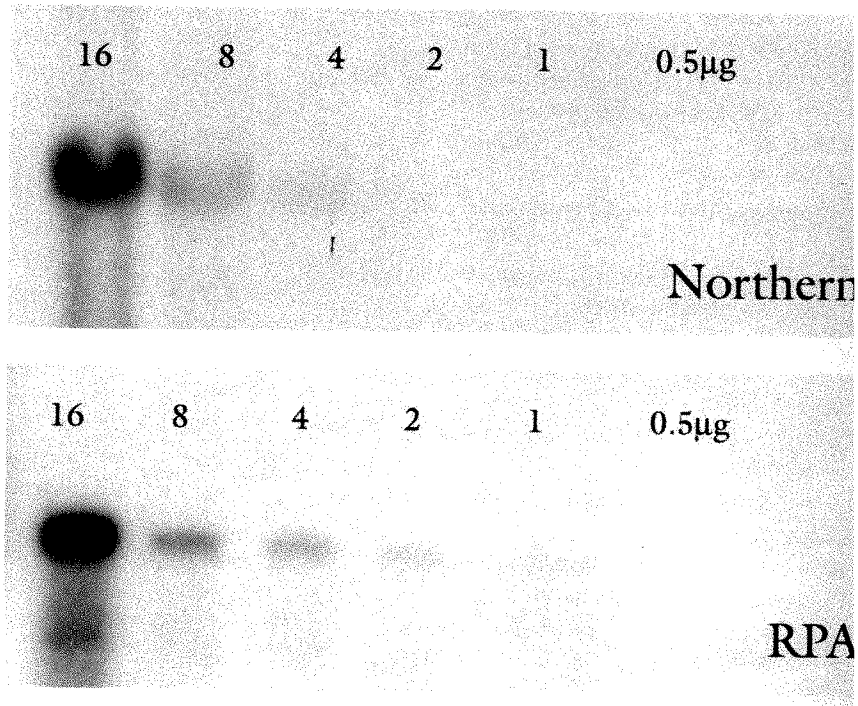

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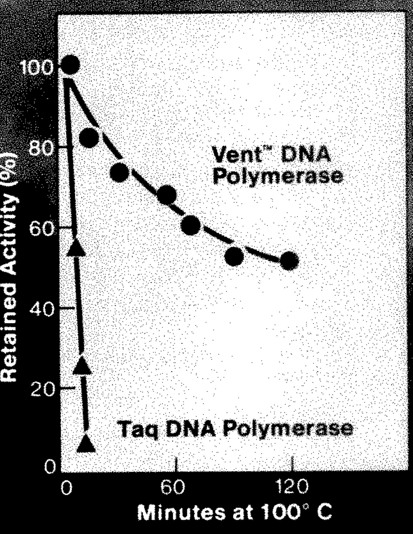

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In vivo imaging, infectious disease diagnostics, and gaining approval of new diagnostics as regulators tighten rules on "substantial equivalence".

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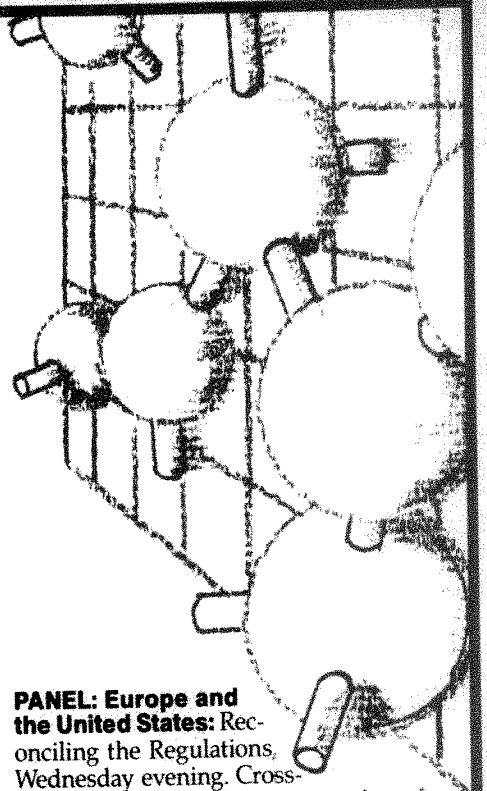

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NE MANIPULATION AND ANSFORMATION SYSTEMS
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os on updating the lab workhorse to ovide faster, more consistent results.
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The publishers of Animal Rights Reporter detail what to expect from the Animal Welfare Act. A panel on anti-activist tactics and facility security will follow.
CHAIR: Mary Lou Sapone, Perceptions Press

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Though proposed regulations have been dropped, this issue won't go away. Could you or your company be vulnerable?
CHAIR: Steven Bongard, Director, Forum on Drug Development and Regulation, Institute of Medicine, National Academy of Sciences

CAREER DEVELOPMENT
How to find — and be — the right person in the right place for the fast track.
CHAIR: Janet Tweed, Principal, Gilbert Tweed Associates

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Pointers on technology management in academic settings: what is worth protecting, how to balance the need to publish vs. the need to profit, and more.
CHAIR: John Wetherell, Attorney, Spensley, Horn, Jubas and Lubitz

LICENSING: OPPORTUNITIES AND SOUND PROCEDURES
Vantages of academia, corporations, and government examined, along with the tax implications of licensing technology.
CHAIR: John Wetherell

PANEL: Europe and the United States: Reconciling the Regulations, Wednesday evening. Crossing the ocean to find new markets for biotech products can be burdensome. Learn what's being done to harmonize the rules so that running the regulatory maze once will be enough.
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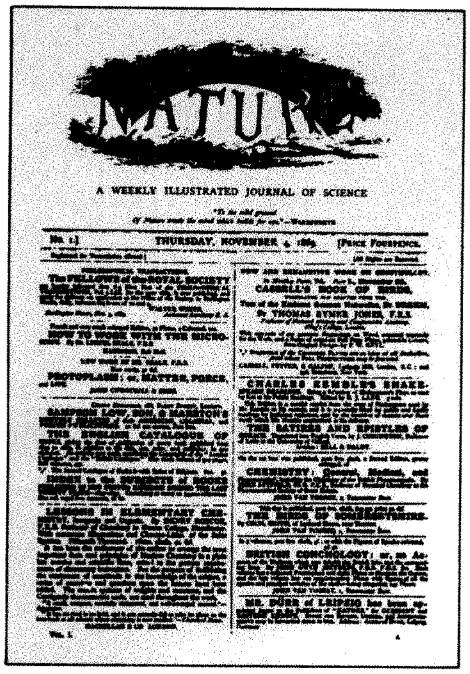

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NATURE VOL 346 nature 26 JULY 1990

The post-Marx dearth of capital

The collapse of communism, the antithesis of capitalism, in Eastern Europe, and its economic failure in the Soviet Union, has brought to light a paradoxical circumstance — the world is dangerously short of capital.

KARL Marx, in retrospect, had a very simple view of life. People would work for the benefit of their communities, benefitting personally, like all others, to the extent the community saw fit. In stalinist times, marxism was an excellent recipe for what would elsewhere have been called capital formation. It was simply necessary to adjust the ratio of those employed to build factories and on other long-term projects to those employed to provide the community as a whole with the necessities of life. The growth of heavy industry in the Soviet Union during the 1930s was a remarkable proof of the efficacy of the process. One of the reasons why communism has come unstuck is that too little was left over to satisfy the legitimate aspirations of the people for a better life. (The accompanying lack of liberty is something else again.) It is a savage irony that most of this past capital investment is inappropriate to modern needs: it has been wasted.

So Eastern Europe and the Soviet Union are on the look-out for more capital, but at the least auspicious time. Most other places are also crying out for the same stuff. Where to find it, and how to recognize it when you do? Capital is nothing but money, and thus indistinguishable from what people use to buy train tickets or dishwashers. The difference is that capital is the money left over after people have spent what they wish to spend on their immediate needs and have paid the interest on their debts. At the personal level, capital is a person's savings. At the national level, the maximum indigenous rate of capital formation is the aggregate of these savings less the net cost of keeping the government in business. People, businesses or governments needing more capital than they have saved must borrow it from the banks and the other agencies of what is now an international market in capital.

Lip reading

The obvious difficulty, for Eastern Europe and the Soviet Union, is that the natural sources of capital are in dire need of the stuff themselves. In the United States, President George Bush has had to go back on his memorable election promise that there would be no new taxes (devices for enforcing savings) in the hope of an agreement with the Congress that the annual budget deficit will be reduced by two-thirds, or by a cool $100,000 million. In Britain, where the personal savings ratio has also fallen dramatically in the past decade, the hapless Minister of Transport, fresh from ruling that the country cannot afford a high-speed rail link to the Channel Tunnel or even a new subway line across London, let slip at the weekend that it cannot afford a new aircraft runway in South-East England either.

But not everybody is strapped for capital. In several countries, Japan and West Germany particularly, both the personal and the corporate sectors are saving hard, while corporate sectors elsewhere have money left over after paying dividends to their shareholders. Where does that finish up? During the past decade, it has been fashionable to invest in the United States, sometimes by buying companies outright (to the frequent chagrin of the locals), sometimes by lending directly to the US government. The reunification of Germany will seriously disturb that habit. The Bonn government, for example, estimates that reunification will cost it DM80,000 million in 1991, most of which will be borrowed from the banks. German companies will invest several times as much in the East. What will be left over for other people?

That is where the crunch comes. When capital is scarce, its price increases, which is why interest rates are likely to remain high for a long time. Outside the umbrella of united Germany, Poland, Czechoslovakia and Hungary will find themselves paying through the nose for private investment — and may consider themselves to have been exploited as a consequence. The imaginative idea of a European investment bank, due soon to be a reality, should blunt the edge of that discontent, but not by much. There will be two losers — the United States, where the economy seems nicely poised between boom and recession, and the poor countries of the world, which will be now more than ever dependent on government-backed assistance, either bilateral or through institutions such as the World Bank. Either way, it is a dangerous prospect for the rest of us. □

Genome goes cool

The human genome project deserves a better hearing from the US Congress than it is likely to be given.

THERE is no good reason why the US House of Representatives should have soured in its view of the Human Genome Project. It would be different if the House were zealously cutting every possible budget item so as to bring the federal deficit under control, but that is plainly not the case. The Senate may be able to insist that the project should not be made to rub along, in the year beginning on 1 October, on roughly half of what it needs from the National Institutes of Health, but it is not well placed to make that point (see page 309). The excuse that the earmarking of research funds for a particular project undermines the primacy of the principal investigator sounds like a deliberate misunderstanding; that the project would consist of a great deal of production-line research work has been made clear from the start.

The essence of the case for the genome project is that the sequence of the human genome will not be compiled by accident. Despite the speed with which particular human genes are being identified and their nucleotide sequences established, the indefinite continuation of that process will never yield the nucleotide sequences of the apparently boring bits of the genome that lie between identified genes, whose evolutionary significance may nevertheless be great. And there is no prospect that even the present rate at which human genes are being identified will make the human genome the analytical tool it might be — a databank of which it is possible to ask interesting questions. Unless the compilation of that databank is commissioned, it will never exist.

That is the case against the position taken by the House Appropriations Committee, but it should be acknowledged that the genome project's own posture is not without its inherent problems. It is caught in a dilemma created by its own existence. The prospect of a deliberate effort to sequence the human genome has stimulated a plethora of new techniques, further complicating the development of a coherent strategy. Sensibly enough, the project plans to cut its teeth on the sequencing of a smaller genome, while constructing a physical map of the human genome. The plan to use NIH money to commission some centres now will give the project the luxury of choice at a later stage, when the responsibility for the long haul will have to be shouldered by a subset of them. Congress's long experience with military projects should be a warning against parsimony at this early stage. But that is a less clear message than those to which congressmen are used.

The military analogy can be taken at least one step further. Making a telling case to the US Congress is a matter of giving the impression that the objective is nicely crystallized without inhibiting the freedom to change course radically at a later stage. Generals pleading for funds for exotic new weapons systems have learned to give the Congress the impression of always knowing exactly what they want; if they later change their minds, they usually manage to get away with it. One consequence of what is called the peace dividend is that there will be experienced Pentagon negotiators out of work. It might suit the genome project's book to hire one or two of these experienced operators to get the appropriations bill through Congress. ☐

Television violence

Evidence that television breeds violence remains as elusive as ever.

WHETHER or not television has the inherent power to incite violence in its consumers, the square-eyed hordes seem to have more personal and cultural 'fortifications' than is usually supposed. The annual research review of the Broadcasting Standards Council, published last week, leapfrogs the debate in Britain over whether the content of television should be regulated, calling instead for a general and dispassionate recognition of the complexity of the issue and, crucially, of the viewer.

The report, the council's first since it was set up in 1988 on an ominous 'pre-statutory' basis, takes a refreshing approach to what has become a rather turgid issue. Instead of trying to account for the role of television in the violent behaviour of violent criminals, the council's researchers, led by Dr David Docherty of the London School of Economics, has asked how television may affect the attitude of the viewing public towards violence itself.

For their main survey, the researchers asked just over 1,000 British viewers how they felt about violence in Britain today — a survey which, as well as providing a useful gauge of public sentiment, allowed the researchers to test for correlations between people's television consumption and their overall attitude towards violence. For instance, do heavy television consumers feel differently about 'legitimate' violence, such as capital punishment and armed police action, than their more abstemious counterparts, and are they, in general, less secure about their own safety?

For the most part, the research shows no significant differences between the heavy viewers and the light on such questions. In fact, the issue that provokes the clearest discrepancy between the two groups — the arming of police in various confrontational situations — shows heavy television viewers to be cooler-blooded in every instance than their library-going and apple-bobbing compatriates. The report concludes that there is a qualitative sifting, on the part of the viewer, of violent images into different categories of palatability. Docherty suggests that it is the extent of the viewer's involvement in the fiction that is the determining factor in these classifications. Television violence that somehow involves the viewer — whether by eliciting an intellectual response or simply by reflecting life in Britain — is far more likely to upset than that which makes fewer claims to realism. In short, the report holds that the context of fictional violence is at least as important as its substance when it comes to earning the label "unacceptable"; and, whether or not television has the power to incite people to violence, viewers are more actively discerning than some proposed legislation gives them credit for. The research does not provide justification for the restrictive regulatory regime that some would like. ☐

First UK trial of AIDS vaccine approved

- First step is cautious but safe
- Twenty volunteers to test prototype

London

THE first British trial of a prototype AIDS vaccine was given the go-ahead by the UK's Medical Research Council (MRC) last week.

The phase I trial, which will be carried out under the aegis of the MRC's AIDS-related research programme, is to begin in September at London's Hammersmith Hospital, where a candidate vaccine known as p24-VLP will be administered to 20 healthy seronegative volunteers. Their immune response to the vaccine will be monitored for up to a year before a decision about further trials is made.

Developed by Oxford-based British Biotechnology Limited, p24-VLP consists of a fragment of an HIV protein known as p24 presented on the surface of a newly-emergent vaccine carrier known as a virus-like particle (VLP). Although not infectious, VLPs possess some of the basic elements of retroviruses, and can be prepared easily in a pure state from genetically engineered yeast cells. Researchers at BBL have shown that VLPs are able to carry proteins that induce HIV-specific immune responses in animals.

News of the trials comes almost three years after the start of the first clinical trial of a prototype AIDS vaccine in the United States. Jane Cope, who is orchestrating the MRC's AIDS vaccine strategy, says that the MRC "didn't want to rush into human studies" without first laying the foundations of a well organized trial system.

But aside from caution, there may be other reasons why the announcement of the first UK trial has been slow in coming. One factor, according to Alan Kingsman, director of research at British Biotechnology Limited, is the dearth of small biotechnology companies in Britain: "The big companies haven't been keen to take it [vaccine development] on", he says. Jonathon Weber of the Hammersmith Hospital, who will be in charge of the clinical trials, notes that most US trials have focused simply on the response of the human immune system to large quantities of crude HIV proteins or peptides. The British trial, however, has a different emphasis: to test the feasibility of using VLPs as novel carriers for 'immunogenic' HIV peptides in humans.

Caution is also evident in the choice of p24 as the vaccine's immunogenic component. Most vaccines tested so far have used the HIV envelope proteins gp160 and gp120, on the grounds that these proteins are exposed on the surface of the virus and so should be strongly immunogenic. In the past, however, questions have been raised about the safety of envelope-derived vaccines, which some researchers have suggested could act to suppress the immune system. No such concern has been expressed over p24, largely because, being a core protein of HIV, it does not participate directly in the virus's interaction with T cells.

Kingsman and Weber agree that p24 alone is unlikely to provide protective immunity against HIV. They view the trial as the first step towards developing a vaccine in which VLPs carry a 'cocktail' of HIV immunogenic protein fragments. The p24 protein is known to stimulate helper T cells in animals and so might play a part in creating protective immunity.

Although trial voluteers will produce antibodies to HIV, the MRC has given assurances that no one will be mistakenly thought to have HIV infection, and so volunteers should have no difficulty obtaining life assurance or mortgages on medical grounds.

The researchers were coy about how they intend to recruit volunteers, but it is unlikely that they will need to call on members of the public. **David Concar**

COLD FUSION

Second round

Washington

FIFTEEN months after the appearance of their first paper announcing the discovery of "cold fusion", Martin Fleischmann and Stanley Pons have published a second paper, 56 pages long, in which they give a full account of the electrochemistry and calorimetry behind their claim that electrolysis of heavy water with palladium cathodes generates more heat than conventional chemistry and physics can explain. The paper, written by Fleischmann, Pons, M.W. Anderson, L.J. Li and M. Hawkins, all of the Department of Chemistry at the University of Utah, appeared in the 25 July 1990 issue of the *Journal of Electroanalytic Chemistry and Interfacial Electrochemistry*, the same journal in which a short paper by Fleischmann, Pons and Hawkins appeared on 10 April 1989.

According to an announcement by Elsevier Sequoia, the journal's publisher, the new paper addresses only questions of electrochemistry, and contains no information on the emission of nuclear particles or radiations which would be expected if "cold fusion" reactions are indeed occurring. The accuracy of the calorimetric results is demonstrated, Elsevier says, by comparisons of palladium–heavy water cells with control experiments using palladium electrodes in light water and platinum electrodes in heavy water. In the absence of direct evidence for nuclear processes in the cells, the implication that fusion is responsible for the excess heat is drawn "by elimination". David Lindley

Light speed — a solar-powered car leaves engine troubles behind on its way to fourth place in the General Motors Sunrayce earlier this month. The 1,641-mile race for vehicles powered by sunlight was won by an entry from the University of Michigan. (AP)

BRAIN RESEARCH

Heading for the nineties

Washington

US neuroscientists' attempts to turn a Congressional resolution designating the 1990s the 'Decade of the Brain' into a financial reality gained a little help last week when US president George Bush signed a proclamation calling on the decade to be observed with "appropriate programmes", and the First Lady, Barbara Bush, opened a two-day meeting intended to publicize the start of the decade.

Both the National Institute of Mental Health (NIMH) and the National Institute of Neurological Disorders and Stroke (NINDS), the two institutes of the US Department of Health and Human Services most likely to benefit from increased efforts in neuroscience, have already released plans laying out what could be achieved in the decade.

The report from NINDS, released last month, also reveals what the decade might cost — for NINDS alone an initial increase of $221 million above the administration's $500 million budget request, followed by a further rise of similar size two years from now.

Funding requests on this scale need a compelling argument if they are to make it through Congress. At last week's 'Decade of the Brain' meeting at the National Academy of Sciences, the logic came in two parts. At the meeting were a selection of the finest neuroscientists working in the United States, speaking in a programme that ran from work on ion channels to neural-network computing. These scientists delivered the first part of the message — that neuroscience is now a mature discipline in the sense that it contains areas in which it is fairly evident what needs to be done and, broadly speaking, how to do it. What that means is that Congress and president can be sure that increased investment will translate directly into increased results.

Areas where the prospects for rapid progress are good were listed by NIMH in a document given out at the meeting: they include the identification and cloning of the genes responsible for schizophrenia, manic-depressive illness, Alzheimer's disease and other mental illnesses; the detailed mapping of where various neurotransmitters and receptors act within the brain; the use of computer-analysed brain activity measurements in improved diagnosis of brain disorders, and use of models of receptor structure to design and synthesize drugs that could help treat conditions, such as schizophrenia, that are thought to be to due to defects in neurotransmission.

The second part of the logic, presented most graphically by Lewis Judd, director of the National Institute of Mental Health is that mental illnesses are a gigantic burden for the health-care system. Judd points out that some 15–20 per cent of people are affected by some form of mental illness each year. The Congressional proclamation estimates that in the United States, the "treatment, rehabilitation and related costs of disorders and disabilities that affect the brain represents a total economic burden of $305,000 million annually". Investment in basic research to produce more effective forms of treatment thus has the potential for generating a large return.

"If you combine the costs of 50 million people a year suffering from mental disorders with a science that is growing exponentially then you have a potentially convincing argument that we're hoping will carry the day, and that funding will flow from it", says Judd. He and his fellow institute directors are not themselves permitted to lobby for increased funds; the job, he says, will have to be performed by the many patient groups (the Cerebral Palsy Foundation, the Epilepsy Foundation and so on) as well as the various professional and scientific societies. A first move of this kind was announced at the conference by David Mahoney, a director of the Dana–Farber Foundation. The foundation is to set up an institute to lobby for support for the decade, and a $25,000-a-year prize to be offered each year of the decade to an outstanding neuroscientist.

Are president and Congress going to be persuaded to support the decade? The House of Representatives has already given mention of the Decade of the Brain as an "area of special concern" in its 1991 appropriations bill for the Department of Health and Human Services, but there will be very little new money available this year. A panel discussion at the conference which included Allan Bromley, White House Science Advisor, James Mason, Assistant Secretary for Health, and Frederick Goodwin, administrator of the Alcohol, Drug Abuse and Mental Health Administration, raised few hopes that riches were about to be showered on neuroscientists. Although steps are being taken to set up a coordinating committee within the White House, most talk at the conference was of the gloomy prospects presented by the growing federal budget deficit.

The next public event in the Decade of the Brain will be the start of a Library of Congress lecture series sponsored by the National Institute of Mental Health. The lectures will continue throughout the decade, with clusters of six lectures each autumn and spring. The first lecture, appropriately enough, will focus on the cost to the United States of brain-related disorders. **Alun Anderson**

DRIFT-NET FISHING

Pyrrhic victory in South Pacific

Sydney & Tokyo

Under pressure from Australia and other South Pacific nations Japan announced last week in Canberra that it will suspend drift-net fishing in the South Pacific a year earlier than required by a United Nations resolution. But the decision will have little overall effect on Japan's drift-net operations, which are based largely in the North Pacific.

Drift-net fishing, which employs sets of nets up to 60 km in total length to catch tuna and squid, has been condemned because it kills other marine life indiscriminately, in particular turtles, seabirds and dolphins. Last year the United Nations adopted a resolution, endorsed by Japan, that bans the technique in the South Pacific from 1 July 1991 and worldwide a year later, unless it is deployed using proven management techniques.

Japan's suspension will come into effect in October, the beginning of this year's season. But, as pointed out by Peter Gill of Greenpeace, "Japan has withdrawn less than thirty boats from the [South Pacific] region while they maintain over one thousand boats in the North Pacific". The South Pacific decision is "chicken feed" compared with the North, Gill says, and he doubts that the UN resolution will bring about a worldwide ban by 1992.

Tania Ewing & David Swinbanks

Wildlife response to the nets

Washington

The US National Marine Fisheries Service (NMFS) finally agreed earlier this month to release some of the data they have collected on the impact of drift-net fishing on marine life after a meeting between Congresswoman Jolene Unsoeld (Democrat — Olympia) and the director of NMFS. Unsoeld has been campaigning for a ban on the technique. The data come from a survey conducted last year by US, Canadian and Japanese observers on board some of the 470 licenced Japanese drift-net boats that catch squid in the North Pacific during the summer months.

Observers looked at a total of 1,402 'sets' of the net — about one fiftieth of the total — in each case examining about three-quarters of the catch. They found that the nets had caught 208 fur seals, 914 dolphins, 22 turtles, 539 albatrosses, 8,536 shearwaters, 25 puffins (an endangered group) and 17 storm petrels. NMFS officials are not yet ready to say what impact this level of mortality might have on the total population of North Pacific sea mammals, turtles and birds. A more detailed survey is being carried out this year with three times as many observers aboard the fishing boats.

Alun Anderson

UK ASTRONOMY

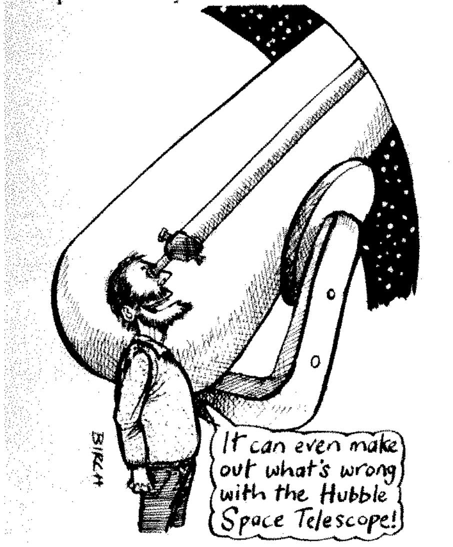
Spanish to step in?

London

THE UK Science and Engineering Research Council (SERC) last week failed to decide whether to collaborate with Spain or with the United States in a project to build what will be the most powerful optical-infrared telescope in the Northern Hemisphere.

But SERC's indecision may now tip the balance in favour of the Spanish collaboration, and is a problem for US astronomers, who need to start their project quickly.

The US National Optical Astronomy Observatories (NOAO) plan to build two 8-metre optical-infrared telescopes: one for the Northern Hemisphere at Mauna Kea, in Hawaii, and one for the Southern Hemisphere at Cerro Pachon in Chile. The National Science Foundation (NSF) can provide only half of the estimated cost of about $170 million, so US astronomers have been seeking foreign collaborators in Britain and Canada.


It can even make out what's wrong with the Hubble Space Telescope!
BIRCH

But Britain has been torn between the US collaboration and a plan to build a Northern Hemisphere 8-metre telescope in collaboration with Spain at La Palma in the Canary Islands. Here, Britain would take the lead in the instrument's construction, basing its design on the smaller William Herschel telescope, also at La Palma.

Britain's main interest is in observing northern skies. A collaboration with Spain would give more observing hours in the Northern Hemisphere, probably at a slightly lower cost, but this must be offset against the better conditions for infrared observation in Hawaii. Both projects aim to have their telescopes ready for use by the late 1990s.

The Spanish collaboration would also allow British astronomers observing time in the Southern Hemisphere. Harry van der Laan, director general of the European Southern Observatory (ESO), says that UK and Spanish astronomers will be able to use ESO's Very Large Telescope (VLT), due to start observation in 2000, provided the arrangement is reciprocal and an adequate system of peer review for one another's projects is worked out. Neither Britain nor Spain currently participates in ESO. The VLT will be a revolutionary instrument, comprising four 8-metre telescopes that can be used independently, or in unison as the equivalent of a 16-metre telescope.

SERC, which has about £20 million to invest, has now deferred its decision until December, after the financial and contractual elements of each project have been scrutinized further. The trend towards closer relations between the United Kingdom and other European countries, both in science and economically, may influence the decision. European collaboration is increasingly seen as being essential to maintain competitiveness with the United States and Japan in 'big science' projects such as space science and particle physics. Moves towards stabilizing exchange rates between European Communities countries may also favour the Spanish option. In 1987, SERC's finances were badly shaken when the UK subscription to CERN, the European particle physics centre, increased as a result of the falling value of the pound.

US astronomers now find themselves in a difficult position. Sidney Wolff, director of NOAO, says that the US project has to get started as soon as NSF money becomes available, to avoid cost overruns. Important decisions on optical design and staff appointments must be made before December, she says. By the time SERC reconsiders, the potential UK input into the NOAO project will have diminished, making it a less attractive proposition for the United Kingdom. In the meantime, British astronomers will continue to refine the design of the La Palma telescope. The delay should be less of a problem for the Spanish, who have yet to finalize their own financial arrangements.

The British indecision may also undermine Canadian support for the NOAO collaboration, although Wolff is optimistic that this will not be the case. She says that NOAO may consider other international partners, while SERC makes up its mind.

Peter Aldhous

EUROPEAN SCIENCE

UK science is sold short

London

THE Treasury's system for handling UK contributions to the European Communities (EC)'s Framework research and development programme compounds the under-funding of British science, according to a report from the House of Lords EC committee, published this week.

Under EC law, member states' contributions cannot be met in full simply by diverting money from domestic spending: there must be some 'additionality' to the EC budget. But the Treasury allows only 30–35 per cent of the UK contribution to Framework as truly additional spending, which the Lords committee says "is clearly far too low". British scientists are successful in winning EC grants, recouping the UK contribution. But Treasury policy means that for every pound of EC research money gained in one year, up to 70 pence is clawed back from the following year's domestic spending on research, by a secretive process called 'attribution' against government departments' budgets.

Attribution has worried the UK research councils, who fear their funds from the Department of Education and Science may diminish as EC spending on basic scientific increases (see *Nature* **345**, 376; 31 May 1990). The report charges that the Treasury has ignored the relatively small UK input in shaping EC research policy, as one member state amongst twelve. Many projects funded by the EC do not coincide with UK priorities.

As EC research spending increases, and attribution eats into the domestic budget, "the [UK] science community's ability to set priorities for projects could steadily diminish", the committee warns.

The report says that the Treasury must be more open about the attribution process and suggests that monitoring by Parliament is needed. The Treasury should also study how other EC states manage their contributions — Treasury officials were unable to explain the arrangements made elsewhere in the EC when asked to do so by Lord Shepherd, chairman of the committee.

The committee's inquiry into EC research and development was prompted by the European Commission's proposal for a new Framework programme, to run from 1990 to 1994, overlapping with the existing programme which finishes in 1992. The peers are satisfied with the new programme's budget, and the balance of funds between different areas of research. But the report says that the Commission needs to take more account of the views of the scientific community in individual member states. This could be achieved by appointing representatives from bodies such as the UK research councils to the EC committees that advise the Commission on science and technology.

Peter Aldhous

- New science minister, page 308.

EPIDEMIOLOGY

Valuable cancer registry

Munich

GERMAN reunification may bring an unexpected boon to the science of epidemiology in West Germany. Unlike West Germany, where strict laws to protect privacy have prevented the accumulation of national records on the incidence of cancer, East Germany has detailed records of all cancer cases since 1953.

West German doctors attending the 'first German medical congress' last month in Dresden spoke out for maintaining the East German registry and for trying to establish more cancer registries on a regional basis in West Germany. Georg Dhom, a pathologist in Homburg/Saar, said that a senior health ministry official had indicated at a meeting last month that the federal government would consider establishing a 'national framework law' to allow the collection of data from cancer patients. Dhom is a member of a health ministry working group concerned with cancer epidemiology.

But as health policy is decided in West Germany by the *Länder* (states), the creation of a framework law could only be a first step. Such a law might encourage the establishment of regional cancer registries similar to those in Britain. West Germany has only two such registries now, in Saarland and in Hamburg.

Even though mandatory registration of cancer cases is forbidden by strict privacy laws in West Germany, Dhom said that the Saarland cancer registry has functioned well with voluntary participation by doctors. Doctors submit data together with the identification of cancer patients to the registry, which removes the names to protect the patients' privacy.

But he criticized as "expensive and technically difficult" the method chosen for a new registry in Baden-Württemberg. There, data are made anonymous before being reported to the central registry; Dohm predicted that double entries will become a major problem. The main reason this method was adopted, he said, was to please data protection activists who fear meddling by the state. But Dhom said that cancer patients in West Germany are in favour of more cancer registries and that opponents have succeeded in preventing their creation by using "very irrational" arguments. **Steven Dickman**

UNIFIED GERMANY

Money, money, money

Munich

THE West German science council Wissenschaftsrat on 11 July called for massive aid for teaching and research in what is now East Germany in order to raise standards and make its universities more attractive to students and professors. At least DM6,500 million (about $4,000 million) will be needed by 1995, exclusive of running costs, said the council.

Good research and advanced study should be moved back from the East German Academy of Sciences to "diverse and properly functioning" East German universities, according to the council.

One way to do this, it suggested, is to remove from the academy the right of granting advanced degrees and teaching qualifications (*Habilitation*).

The council urged East German institutions and Western reviewers to resist the pressure simply to impose the West German system on East Germany. Instead, West Germany should take the opportunity to identify its own weaknesses and correct them.

The council also supported an increase of at least 25 per cent in the budgets of the Alexander von Humboldt Foundation and the German Academic Exchange Service (DAAD) to promote the international exchange of senior and junior researchers as well as students in a united Germany. **S.D.**

NAZI VICTIMS

Graves desecrated

Munich

UNKNOWN persons on 15 July desecrated the graves where body parts and tissue samples taken from the corpses of Nazi victims had been buried 11 days earlier. The samples had been in the collections of the University of Tübingen and were used for research and teaching purposes until a scandal in 1989 prompted their removal and eventual burial.

The criminals spray-painted swastikas onto the gravestones in the Tübingen city cemetery and hacked to pieces the stone that commemorated the victims.

The University of Tübingen held a commemorative ceremony on 8 July in honour of some 400 people whose remains were discovered in the collections of the Anatomy Institute. At the recommendation of an external committee, the university removed from the collections the remains of anyone who was known or even suspected to have been killed by the Nazi regime.

The destroyed stone was carved with a motto written by University of Tübingen pathologist Jürgen Peiffer: "Abducted, oppressed, violated,/Victims of despotism or blinded justice,/People did not find their rest until they arrived here./From their corpses even,/was demanded a service,/by a science that did not respect human values and dignity. /May this stone serve as a warning to the living." S.D.

RADIATION HAZARDS

Data released at last

Washington

THE controversy over whether exposure to low-level radiation is linked to an increase in cancer deaths looks set to be refuelled after the US Department of Energy (DOE) last week finally released data on the radiation exposure and mortality rates of workers at nuclear weapons plants. The data are being released to the Three Mile Island Public Health Group which disputes the the official DOE opinion that low levels of radiation are safe because at worst they are linked only to one very rare form of cancer. DOE officials have until now been reticent to release their data, claiming that information on workers' health is confidential.

Success for TMI in a three-year lawsuit came only after pressure from Congress and the recommendation of a DOE panel of enquiry that public access be given to workers' health statistics.

This first installment of newly released data includes records of the radiation exposure and mortality rates of 44,000 people who worked at the DOE's Hanford Facility in Washington between the 1940s and 1981. Hanford is the DOE's oldest nuclear weapons manufacturing plant.

TMI will eventually have access to data on 200,000 workers, one-third of all of DOE 's personnel. But the terms of the legal agreement between TMI and DOE prevent TMI from releasing this information outside their research team.

With the new data, the 83-year-old TMI epidemiologist Alice Stewart hopes to update her earlier controversial studies with the Hanford population of workers. Her work was stopped in 1977 when her supervisor, Thomas Mancuso, lost his contract with DOE. That decision was in itself the subject of a congressional hearing.

Mancuso and his co-workers had published a study suggesting that increases in many kinds of common cancer could be seen at levels of radiation exposure that DOE considered safe.

Rival epidemiologists, including those under contract with the DOE and independent groups in British and Canada, dispute this finding. According to DOE contract researcher Ethel Gilbert of Battelle Pacific Northwest Laboratory, they have found only suggestions of a correlation between low-level radiation exposure and the ocurrence of multiple myeloma, a very rare cancer, in the DOE worker population. Data collected in Japan from studies of A-Bomb survivors do not support Stewart and Mancuso's conclusions.

DOE officials are currently considering how they will make data on all their workers available to the public. **Robin Eisner**

COMPUTER INDUSTRY

Fujitsu's long march

London & Tokyo

Fujitsu, Japan's largest computer maker, is to bid for a majority shareholding in ICL, the only British company still manufacturing mainframe computers. News of the deal, leaked to the press two weeks ahead of the planned announcement, has sent a shockwave through the computer industry as it is seen as the most daring of a series of moves by Japanese companies determined to build global competitors to the US giant, IBM.

If successful, the bid will take Fujitsu from the number four position, in terms of global revenues, to the number two position, leapfrogging NEC of Japan and Digital Equipment Corporation of the United States. But Fujitsu and ICL's combined revenues for 1989 were still less than a quarter that of IBM.

Fujitsu is expected to bid for at least a 50 per cent holding in ICL at the end of this month. The Japanese company would gain a profitable subsiduary (ICL had pre-tax profits of £145.7 million last year) and easy access to the computer market in the European Communities (EC), when trading restrictions between the EC partners are abolished in 1992. Some link up between the two companies had been expected, since Fujitsu already supplies ICL with semiconductors for its machines. But few predicted that STC, ICL's parent company, would be prepared to give up a majority stake.

Fujitsu's takeover bid is just one of several moves by Japanese computer manufacturers intended to form alliances against IBM with European and US companies. European companies are not themselves seen as serious competitors; with the backing of the Ministry of International Trade and Industry (MITI), Japanese companies are concentrating single-mindedly on overtaking IBM.

Apart from its ICL link, Fujitsu has a major shareholding (43.6 per cent) and cooperative marketing agreement with the US computer maker Amdahl, which uses Fujitsu technology in its computers. The Japanese company also has an agreement with Siemens of West Germany, whereby Siemens sells Fujitsu computers with Siemens labels on them.

Hitachi and NEC have gone into similar licensing agreements and joint ventures in Europe, producing a marketing network for Japanese companies that in total matches IBM's in scale.

UK Opposition Labour party technology spokesman Lewis Moonie says he is "disappointed that the only remaining British mainframe manufacturer has to pass into foreign hands". He blames the lack of a coherent government strategy for information technology for the current state of the British computer industry, and is also concerned about the takeover of the UK software company Hoskyns by the French group Cap Gemini Sogeti, announced last week.

But Martin Campbell-Kelly, who lectures in the history of the computer industry at the University of Warwick, does not view the ICL takeover as the death knell for the British computer industry. Rather, he says, the industry has moved on from the manufacture of hardware to the production of integrated computer systems assembled from hardware manufactured elsewhere.

Campbell-Kelly says that two important questions surrounding the ICL deal remain unanswered. The first is whether Fujitsu will discontinue ICL's mainframe range to concentrate on marketing their own products. The second is what will happen to ICL's research and development base once the company passes into Japanese hands.

Some UK observers predict a diminution of research, but Japanese electronic companies typically re-direct large percentages of their profits into research and development, suggesting that Fujitsu will make full use of ICL's research capabilities. Several Japanese computer manufacturers have already set up research units in Europe and the United States.

Peter Aldhous & David Swinbanks

OCEAN RESEARCH

Japan dreams deep dreams

Tokyo

Japan's ambitions to have the biggest and the best of everything appear to have touched the Ocean Development Division of the Science and Technology Agency, which last week sent in a request for funds to begin work on the design of the world's largest ocean drilling ship.

The initial request is for just ¥97 million ($620,000). But the design is to be for a ship of 18,000 tons (gross) — more than twice the size of the US ship *JOIDES Resolution* which is used in the International Ocean Drilling Program. Yoshiro Miki, director of the Ocean Development Division, says the ship would be equipped with all the most sophisticated Japanese instruments, would cost about ¥30,000–¥40,000 million ($200–$270 million) and would be ready before the end of the decade.

The International Ocean Drilling Program is scheduled to end in 1993. But it may be extended by another five years or so, in which case the Science and Technology Agency will discuss how to share the drilling activities of the two ships with the countries involved in the programme.

Somewhat surprisingly, the Ministry of Education, Science and Culture, which at present supports Japan's participation in International Ocean Drilling Program, has not expressed any opposition to the Science and Technology Agency's plans. The two organizations often fight for territory in basic research but Miki says ocean research is one area in which they have learned to collaborate.

The only thing that might thwart the agency plans to build the drilling ship is the Science and Technology Agency's commitments to space research. The agency's participation in the US space station project will drain its resources over the next decade. But Miki and other agency officials are determined to try to maintain a well-balanced budget for all areas of scientific research.

The Ocean Development Division has other new projects, besides the drilling ship, which it hopes to cover with a total 24 per cent increase in its budget for next year. The budget request, for ¥12,000 million ($80 million), will almost certainly be whittled down by the Ministry of Finance over the coming weeks but is in line with recommendations published by the Council for Ocean Development of the Prime Minister's Office in May and a call for increased research on Earth science and technology issued last month by Japan's highest science policy-making body, the Council for Science and Technology. Miki says he is confident that the new projects will be funded.

Biggest among them is the DEEP STAR (Deep-sea Environmental Exploration Program-Science and Technology for Advanced Research) project, which aims at growing deep-sea microorganisms in the laboratory at pressures up to 650 atmospheres (the pressure at a depth of about 6,500 metres in the ocean). The project began this financial year with a budget of ¥790 million ($5.2 million) to build equipment for collecting microorganisms from the sea floor. A total investment of about ¥4,500 million ($30 million) will be required over three years to complete facilities on land, Miki says.

Koki Horikoshi of the Institute of Physical and Chemical Research (RIKEN), who won international recognition for his 'Superbug' project (see *Nature* **310**, 172; 1984), will head the DEEP STAR project at the Japan Marine Science and Technology Center (JAMSTEC).

The budget request also includes several hundred million yen to study ocean circulation and climate change near the North Pole in collaboration with the United States and West Germany.

David Swinbanks

ANIMAL RESEARCH

Battle lines are drawn

Washington

UNDER pressure from both animal welfare groups and scientists, two groups with widely divergent views of how the government should regulate the use of research animals, the US Department of Agriculture (USDA) last week opted for a compromise; it issued comprehensive new rules on the care of guinea-pigs, hamsters and rabbits, but inserted language to ensure that few laboratories will be forced to buy new cages or make other expensive changes to conform.

The new rules are the first of two controversial sets of regulations to make it all the way through the regulatory process and to be published in the *Federal Register*. Both have been the subject of a five-year struggle between the agency, Congress and activists. As such, the battle over them has been mostly one of principle; the guinea-pig and rabbit regulations are seen as a precedent-setter for the still-unresolved and much more contentious regulations for non-human primates and dogs (see *Nature* **345**, 755; 28 June 1990).

At issue is the concept of "performance-based" versus "engineering" regulations. Scientists argue that they should be given some leeway to design animal caging that does not exactly conform to some minimum size standard as long as they can show that the animals are healthy and happy. Animal welfare lobbyists, on the other hand, say such performance-based regulations are unenforceable, and that anything other than strict size standards are no standards at all. The Animal Legal Defense Fund, which has sued USDA to release the primate and dogs rules, is considering a lawsuit over the guinea-pig and rabbit regulations as well.

Although the new guinea-pig and rabbit regulations lay out strict cage-size minimums, they are "grandfathered" — they apply only to new caging bought after the rules take effect next month. More importantly, the rules allow an exemption for "innovative enclosures that do not precisely meet the space requirements". What that means is that good cages are not necessarily standard boxes, says John Miller who, as director of the animal welfare office of the National Institutes of Health (NIH), helped to write the new regulations. Because, for example, hamsters prefer walls to open spaces, if one were to design a hamster cage that was a series of interconnected tubes, which maximize the wall area but may not quite meet the minimum floor space requirement, the enclosure would still be perfectly acceptable, Miller says.

"That's precisely why the 'innovative enclosures' clause was inserted — to give the regulations that performance flavour". Miller explains. But he stresses that the clause does not offer *carte blanche* for scientists to ignore the size standards. "Smaller than minimum does not constitute innovative", he says. At any rate, because the new regulations put into law the NIH guidelines that have been in place since 1972, almost all NIH-funded animal laboratories are already in conformance, Miller says.

The still-unfinished rules for primates and dogs are now likely to also include an similar "innovative enclosure" clause, according to Miller. Since the White House rejected the USDA's previously proposed engineering-based standards earlier this year (see *Nature* **344**, 804; 26 April 1990), the agency has retreated to lick its wounds and rewrite the regulations in performance-based language. It intends to resubmit them for review and public comment this fall.

G. Christopher Anderson

US SPACE PROGRAMME

Stone voyages to JPL

Washington

VOYAGER 2, the phenomenally successful interplanetary probe whose flyby of Neptune last year provided a much-needed boost to the National Aeronautics and Space Administration (NASA) space programme, has left yet another success in its wake. Edward Stone, Voyager's scientific director, has been selected as the next head of NASA's top planetary and robotics facility, the Jet Propulsion Laboratory (JPL). Stone will replace Lew Allen, who is retiring in December.

Stone will be taking over a laboratory that has seen much of its original robotics mission eroded by an increasing NASA emphasis towards manned space exploration. He says that broadening JPL's base beyond planetary science — a process already begun by Allen — will remain a top priority. Likely areas of growth include work on parallel-processor supercomputer, radiation-hardened electronics, robotics and instrument-building for other NASA laboratories. Further changes at JPL — including a possible increased emphasis on missions focusing on cosmic particles and fields, Stone's scientific specialty — may be some time off.

JPL's development over the next two years has already been set and any new direction at the laboratory will have to wait, Stone says.

G. Christopher Anderson

FRENCH BUDGET

Research still in favou

Paris

**THE French government has once agai
made education and research top prioriti
in its annual budget. Although ministe
have been told only the global figures th
can expect — it is up to them to propo
how the money should be spent — educ
tion is set to receive 9 per cent more th
last year and research and technology
increase of 7.5 per cent. Inflation in Fran
is currently 2.5 per cent.**

**The national education budget, whi
includes university research, but not oth
state research money, will increase
FF20,000 million ($3,610 million)
FF250,000 million. The increase will
swallowed up by a major new constructi
drive within higher education and am
tions to create thousands of new posts in
sectors of education.**

**Unlike most of its neighbours, Fran
will not decrease its defence budget ne
year. Jean-Pierre Chevènement, the defen
minister, has apparently succeeded
fighting off proposals to cut his budget a
has even won a 3 per cent increase, ju
ahead of inflation. But greener issues a
still close to President François Mitterrand
heart: the environment ministry will see i
budget up by 15 per cent in 1991.**

**Ministers and civil servants now have t
summer to prepare detailed budgets in tin
for a cabinet meeting in September.**

Peter Col

UK RESHUFFLE

New minister for old

London

**ROBERT Jackson, UK minister for scien
and higher education, has been mov
from the Department of Education a
Science (DES), as part of a reshuffle
middle-ranking ministers designed
improve the Conservative government
image and performance in the run up to t
next general election. Jackson had be
widely criticized for his piloting throu
parliament of a bill introducing loans f
students, and the failure to convince t
major banks that they should administ
the scheme. More recently, he was attack
by colleagues after joking at London
Heathrow airport that his ministerial b
contained a bomb.**

**Michael Fallon, newly appointed to t
DES, is expected to take over Jackson
responsibilities. The British scientific co
munity will hope that Fallon's appoin
ment introduces a more sympathetic att
tude from the DES towards the need f
increased funding and improved care
prospects in UK science. Jackson h
maintained that the UK 'brain drain' is
myth, and was labelled "the rottweiler
academia" at recent press conference
the pressure group British Scientis
Abroad.**

Peter Aldho

HUMAN GENOME PROJECT

The honeymoon is over

Washington

AFTER an infancy of accolades from politicians and scientists alike, the Human Genome Project is finding that growing up is hard. The House of Representatives Appropriations Committee last week cut the project's 1991 funding to $66 million — nearly $34 million less than the president's request, which was itself $24 million less than genome project officials had originally asked for. Overall, the House figure is about one-half of what genome project planners had hoped for next year and reflects a substantial erosion of confidence in the project among legislators.

House Appropriations Committee staff say they are concerned that the project, which is orientated toward centres rather than individual grants, would contribute to the trend at the National Institutes of Health (NIH) away from support of single investigators. They also say that project officials have made a poor case for their budget request, by providing detailed numbers only when asked and by lifting much of the request from the recommendations of a 1988 National Academy of Sciences report.

Staff say that although a letter-writing campaign by scientists opposing the project was not a major factor in the House decision, appeals from other researchers in support of more individual-investigator grants were persuasive.

NIH genome centre director James Watson says the House has missed the point. "I think they've misunderstood the role of centres in the project", he says. Much of the sequencing and mapping of the project is routine work best suited to a small number of well equipped laboratories. Although fundamental research is often well suited to small laboratories and single researchers, the genome project requires something closer to factories. "Congress needs to make the distinction between exploratory research and getting the job done", Watson says.

Legislators on the Senate Appropriations Committee side with Watson on the initiative. But although they say they will try to vote the president's full $108 million request, the vagaries of the budget process have left the committee with nearly $2,300 million less for NIH as a whole, and it may be forced to make cuts as well.

Once the Senate has arrived at a figure, the two arms of Congress will negotiate a compromise budget in conference. Short of a catastrophic across-the-board cut to satisfy the Gramm–Rudman deficit-reduction provisions, the genome project is likely to get something between the House and Senate figures and far less than the president's request.

Officials at the genome project's NIH headquarters are not counting on a substantial reprieve. Three new projects that were about to be announced — a new pilot sequencing programme, a mapping database and a project to locate index markers on the genome — are now in abeyance until the picture clears, says genome office budget officer Erin Burgess. NIH had also planned to start nine new centres at about $4 million each in 1991; the House cuts could lower that to as little as three centres at $2–$2.5 million each.

Funding for the three projects now in limbo was to have amounted to about $19 million next year — just a little more than the $18 million that Congress has placed tantalizingly out of reach in a special discretionary fund for the director of the NIH. This fund can be made available to the genome project at the discretion of the NIH director, once a director has been appointed. There has already been a delay of nearly a year in filling the position, which has caused increasing tension between NIH and Congress. House staff say that they want a new director soon, and they want that director to take a new look at the progress and management of the genome project.

Genome centre officials fear that linking the genome centre's fortunes to the arrival of a new NIH director places the project's growth in jeopardy. "We're not feeling very positive about the money. We're hoping the Senate will say you can't hold the genome project hostage for a new director", says Burgess.

The Department of Energy (DOE), which runs about one-third of the project, has so far done much better in the appropriations sweepstakes. DOE funding from the House is $46 million, just what the president requested.

G. Christopher Anderson

ABORTION PILL

French pill for Britain?

Paris & London

RU-486, the abortifacient pill, could be marketed in Britain by next year if its manufacturer, the French company Roussel-Uclaf, goes ahead with its decision to apply for a product licence. At present, says the company's head of marketing, Ariel Mouttet, Roussel-Uclaf is making enquiries to ensure that the strict controls surrounding distribution of RU-486 in France can be maintained in Britain. If so, the company will send its application to the Department of Health.

At present, RU-486 is only available in France. Even there, it took state intervention to put the drug back on the market after it was withdrawn following threats to company executives from anti-abortion groups (see *Nature* **340**, 6; 1989). Only now, after successful clinical trials in Britain and over two years of use in France, is Roussel-Uclaf preparing its first major attempt to market the drug abroad.

The drug already has some supporters in British medical circles and, according to Mouttet, Kenneth Clarke, the health secretary, has written to encourage Roussel-Uclaf to apply for British registration. Naren Patel, honorary secretary of the Royal College of Obstetricians and Gynaecologists, says that "preliminary reports seem very favourable", although he stresses that he has not yet seen all the data on clinical trials. Patel believes that the drug may be more appropriate for certain terminations than existing techniques. A spokeswoman for the British Medical Association was also supportive. "We do not see any immediate medical problems", she said. But she pointed out that abortions still require the consent of two doctors, under the terms of both the old Abortion Act and the Human Fertilization and Embryology Bill, currently before parliament.

Roussel-Uclaf is also keen to dispel any ideas that RU-486 will be available over the counter as a 'morning after' pill or for 'do-it-yourself' abortions. In France the drug is available only within approved clinics and hospitals and must be taken in the presence of a doctor, in association with synthetic prostaglandins.

Mouttet explained that the company will not proceed with its application unless tight distribution controls are respected. In France the drug is treated in the same way as are opiates and controlled narcotics. At pharmacies it must be kept in a locked cupboard and a complex system of numbered order books, consent forms and sticky labels ensures that every box of the drug can be accounted for.

An estimated 40,000 women have now aborted using RU-486 and Roussel-Uclaf has full records on 20,000 of them. A study of the first 2,000 cases has been published and a report on a further 10,000 women is available within Roussel-Uclaf. Until March this year, the drug was issued free and, says Mouttet, orders were running at "about 4,000 boxes per month". Now there is a charge, demand has dropped to "about 3,000 a month".

If RU-486 is marketed in Britain, the manufacturers will next consider approaching Scandinavian countries. But Mouttet confirmed that the company still has no plans to market RU-486 in the United States or to carry out clinical trials there. If the drug is easily available in Britain, however, a black market for the drug might easily develop in the United States.

Peter Coles & Peter Aldhous

Send Hubble to the Moon

SIR—The proposal of Bahcall in *Nature* of 22 June last year[1] to establish an international institute for astrophysics is an important one. In particular, their suggestion of taking to geosynchronous orbit the 2.5-metre test mirror developed for the Hubble Space Telescope (HST) programme is an interesting possibility.

This is an example of reducing astronomical costs as well as sharing them. In low Earth orbit, the HST must be serviced during the next solar cycle, which may be a costly exercise, possibly compromised by the flight availability of the Space Shuttle. In geosynchronous orbit, on the other hand, an HST instrument would need less servicing, and would provide direct data access (see ref. 2 for a discussion of 90-minute operations). It would have direct command and control, three times the efficiency of observations, and ambient-cooled optics (being further from Earth).

I would like to take this suggestion one step further, and point out that the Hubble test mirror could be taken to the Moon, again as part of a cost-effective strategy. NASA has recently conducted three workshops[3-5] addressing physics and astronomy from the Moon, the first of which contributed to a presidential decision that a return to the Moon would be the next major goal of the US space programme. It is clear that the Moon, as our nearest neighbour, offers distinct advantages for space astronomy over low-Earth orbit and geosynchronous orbit, and over any planetary initiative.

Taking the Hubble test mirror to the Moon, at the onset of the programme, could serve as a focal point for the lunar initiative, placing the programme's emphasis on science and astronomy. In this scenario, the test mirror with modern instrumentation and redesigned actuators for a structural 1/6-gravity environment at a lunar base (rather than zero-gravity in Earth orbit) would be put in place immediately at the landing site on the second or third manned mission to the Moon. (There may be unmanned excursion missions, landing before the manned phase.)

A 10,000-pound Hubble instrument is 4.5 metric tonnes, one of the very payload weights currently used by NASA's Office of Exploration[6] in its preliminary models for the manned flights there. With the approval of the US Congress, one can easily imagine the Hubble 2.5-metre mirror at a man-tended lunar base within this decade (by the year 2000, one of the schedule dates now in use[6]).

The existing proposals[3,5,6] on astronomy from the Moon have been described as "lacking vision". Using the Hubble test mirror in this way, that is, putting it in place at the onset (the second lunar landing) at low cost (as an 'off-the-shelf' instrument) could catch the public imagination and muster the scientific support required for a visionary approach (see H.J. Smith in ref. 5 for discussion). This must not compromise the Hubble programme itself, it must be international in scope, and it must be followed by a more ambitious set of observatories using larger, higher-quality mirrors (since the Hubble test mirror does not meet the needs of space astronomy in the twenty-first century).

The Hubble emplacement, early on, would also serve as a testbed for determining requirements and problems for astronomy from the Moon as the initial outpost evolves into a mature lunar base.

THOMAS L. WILSON

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Global warming

SIR—Perhaps you have not received any correspondence that presents a positive or optimistic outlook for the changes that are predicted to accompany or be a consequence of global warming? The good news that large areas of Canada and the Soviet Union may become more conducive to agriculture seems largely discounted. More optimistic speculations could centre on how the results of the greenhouse effect might circumvent the burning of fossil fuels for energy. Apart from the argument that we may need to burn less fuel for heating in a warmer world, going further, desert areas could become the 'oil wealth' of the future if they were turned over to massive-scale production of solar-generated electricity. This abundant electricity might be used to make hydrogen for use in internal combustion engines. Or perhaps there could be solar-powered desalination plants to produce fresh water for use in, say, irrigation programmes. Such schemes would require labour, but might appeal to people forced to move if their homes became uninhabitable as a result of climatic change.

Such an optimistic outcome would, of course, require planning, management and co-operation between nations on an unprecedented scale. Such intergovernmental co-operation may seem unlikely, but an unbalanced, gloomy outlook surely cannot help it to come about. I hope you will encourage the avoidance of doomsaying in such matters.

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Australia prize

SIR—Tania Ewing's story on the Australia Prize (*Nature* **344**, 692; 1990) need correction.

Professor Allen Kerr did not isolate the bacterium responsible for crown gal disease. That was done long ago. He was however, the first to show that pathogenicity in *Agrobacterium tumefaciens* could be transferred from one strain to another.

This transfer was then shown by the groups associated with Professors Eugene Nester and Jeff Schell to involve the large tumour-inducing plasmid (pTi). This was the beginning of the development of an effective plant transformation system, to which their groups have contributed so many advances.

Ewing errs again in attributing to them the genetic engineering of a benign strain of *Agrobacterium* to ensure continuing effectiveness of the system of biological control of crown gall disease. That was due entirely to Kerr's group.

DAVID CURTIS

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Crisis in education

SIR—It seems that there is a dearth of applications for postgraduate research awards this year, but there is a widespread reluctance on the part of supervisors to admit how few applications have been received for the awards on offer, as they fear that the poor application rate will discourage funding bodies from continuing their support.

This is a mistake. Rather than concealing the situation, we should be shouting and screaming about the low number of aspiring postgraduates, as it is symptomatic not of problems within individual departments, but of a major crisis within the whole higher education system that must be addressed now. A dearth of postgraduates now will be a constraint on the future development of research in this country.

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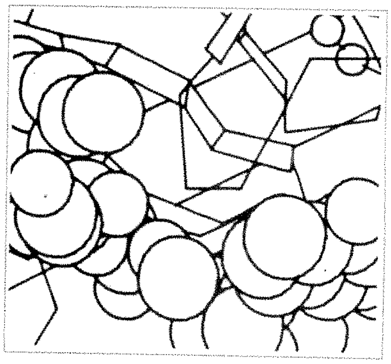

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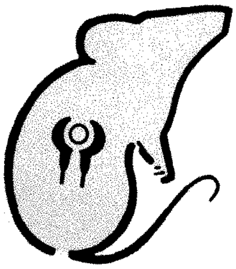

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Christopher Goodnow (Stanford)
Susumu Tonegawa (MIT)

2. ANTIGEN PROCESSING AND PRESENTATION

Jack Strominger (Harvard)
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Alain Townsend (Oxford)
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3. AUTOIMMUNITY AND CONTROL OF IMMUNE RESPONSE

Hugh McDevitt (Stanford)
Leroy Hood (Caltech)
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4. VACCINES AND ANTIBODIES

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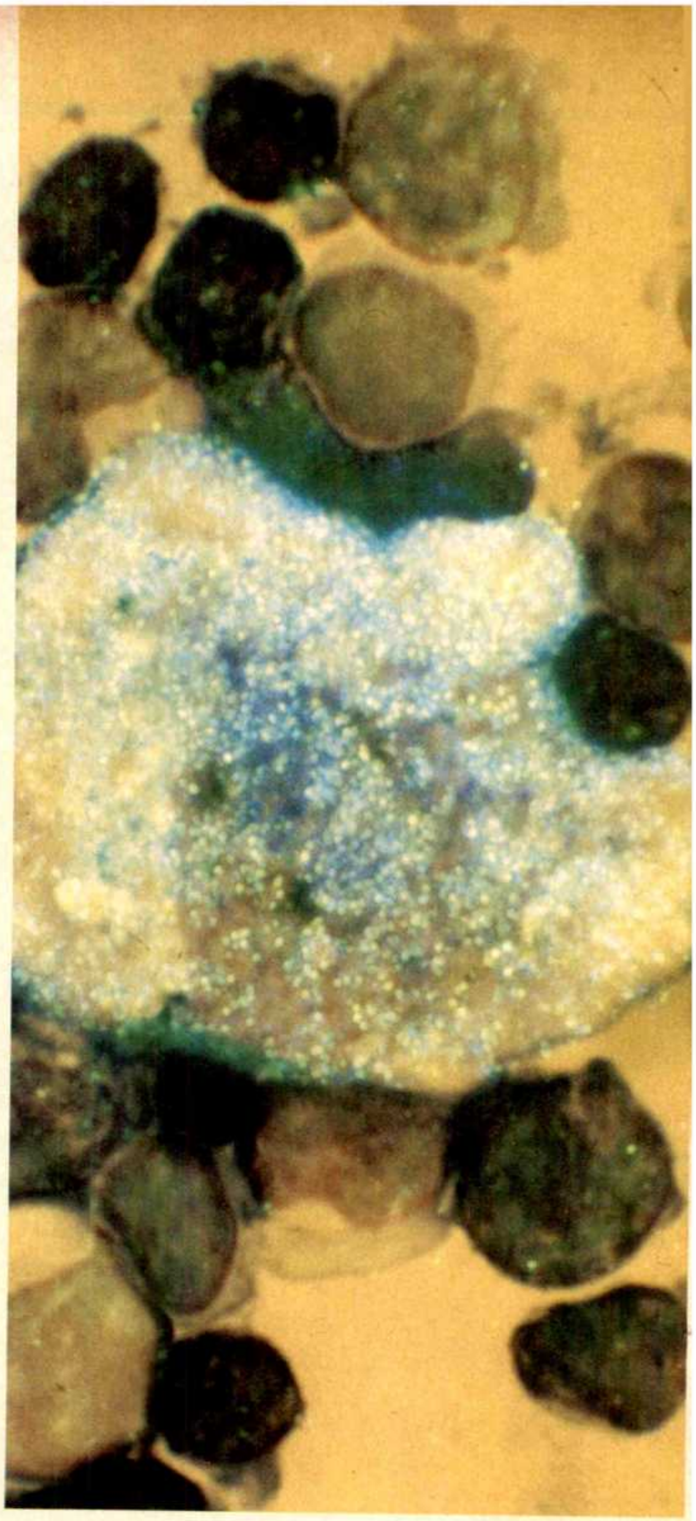

Mononuclear cell suspension of human peripheral blood containing Hodgkin's cells. Courtesy of Dr. M. De Waele, Vrije Universiteit, Brussels, Belgium.

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Can mirrors beat the greenhouse?

People should not personally assume responsibility for the greenhouse effect, which would be impossible, but they should give some thought to the quantities involved.

AMONG the green community, the prospect that the excess greenhouse effect may be a reality, even here and now, has evoked a general wish to be seen to be doing something to assist in its abatement. That is natural enough, even though there are the strongest possible reasons for suspecting that individual virtue will serve only to provide the less virtuous with a licence not to follow suit, which is why the proper regulation of the greenhouse requires an international convention. Meanwhile, a child's guide to some of the steps that individuals might take may also serve as a guide to the underlying simple arithmetic.

At the present average distance between the Sun and the Earth, the solar energy flux normal to the line of sight is roughly 1,370 W m^{-2}, but only some 70 per cent of that reaches the surface of the Earth. (Ultraviolet is mostly absorbed by the ozone layer, which is mostly still there, radio waves are screened out by the ionosphere and so on.) The average sub-zenith solar flux may be taken as 950 W m^{-2}, not very different from 1 kW m^{-2}, but that is only ever nearly valid in the tropics, while half of the surface of the Earth is always turned away from the Sun; the average flux at the surface is therefore one quarter of the sub-zenith flux, say 240 W m^{-2}.

It is important that the mismatch between incoming and outgoing radiation fluxes expected from the accumulation of CO_2 and other greenhouse gases is of the order of 1 W m^{-2}. This, for example, is the degree of what is called in the trade the 'forcing' of the Earth's climate system caused by the accumulation of CO_2 and, to a lesser extent, of methane, between 1850 and 1960 — then the only greenhouse gases worth talking of. (CFCs have become a serious contributor to the problem only since that time.) People bent on personal steps to ameliorate the excess greenhouse effect must keep that figure in mind. What can they do?

First, perhaps, they should consider what might be done with simple mirrors. If one happened to live in the tropics, one might hope that a square metre of silvered glass placed in one's back garden would reflect back to space a substantial fraction of the incident flux of energy — perhaps not the whole of 1 kW, but roughly a quarter of it. But even that is not quite true. When the mirror surface is away from the sub-zenith point, the absorption and scattering of energy on the longer pathways into and out of the atmosphere will be greater. Perhaps the most one can hope for is that an eighth of 1 kW m^{-2} will on the average be turned away.

Naturally it is worse when, like most people, one lives outside the tropics. Then there is an extra factor of cos α (where α is latitude) to be taken account of, as well as the extra absorption and scattering in the atmosphere. At latitude 60°, that means a further factor of two on account of latitude, and yet another factor of two on account of extra path length through the atmosphere. If the albedo of the part of the surface on which the silvered mirror is placed is that of a perfectly absorbing black body (which cannot be exactly true), a single square metre of silvered surface will turn back only an average of roughly 30 W. Carpeting a few per cent of the Earth's surface with mirrors would be necessary to counteract the greenhouse forcing so far (which emphasizes why snow cover and sea ice are important in climate models). But an area smaller by a factor of about four would be required in the tropics.

Absolute quantities are important at this stage. In round numbers, the surface area of the Earth (two-thirds of which is water) is 5×10^{14} m^2, which means that the greenhouse forcing of the past 150 years is the equivalent of 5×10^5 GW of energy — not very different from the world's present production of electricity.

Even if one takes the view that one's personal responsibility is limited to one's fair share, it would still be necessary, if repairing past damage is the goal, to be personally responsible for returning to space the equivalent of 100 kW of solar energy, requiring an investment in some 3,000 m^2 of mirror surface outside the tropics or in something like 750 m^2 in the Sahara. If, on the other hand, one took the view that it would suffice to neutralize by mirrors one's incremental contribution to the excess greenhouse effect, then because the next fifty years are expected to be about as damaging as the past 150 it would suffice to lay 150 m^2 of one's non-tropical back garden with mirror surface every year, or to arrange that an agent acting on one's behalf in the Sahara similarly treats 20 m^2 or so of desert surface every year.

This argument is a *reductio ad absurdum*, and is meant to be. (Most people do not have back gardens that big, in any case.) But the orders of magnitude are important. The calculated imbalance between the incoming and the outgoing radiation is of the order of a few kW per person now alive. It must surely be relevant that most of the 5×10^9 of us do not have the opportunity to consume that much electricity.

The opportunity for a technical fix arises at this point. The green community naturally abhors technical fixes, often on the grounds that they are palliatives rather than permanent 'solutions', but it should not go unremarked that the most economical solution of the greenhouse problem by means of mirrors must be that lightweight mirrors, no doubt made of aluminium foil, should be installed in the line of sight between here and the Sun.

No doubt there would be countless opportunities for endlessly more sophisticated calculations of the effects of radiation pressure on such structures. There have already been several calculations in the past of how such devices might be used to channel solar energy towards collecting stations on the surface of the Earth. Faced with the contemporary version of the problem — how best to get rid of solar energy — the space research community would no doubt be able to rise to the challenge.

That is why the true essence of the greenhouse problem is not the question of whether the concentration of CO_2 in the atmosphere is increasing (it is), or that of the degree to which CFCs are more important as greenhouse gases than CO_2, but that of how the accumulation of these materials in the atmosphere will affect the average temperature on the surface of the Earth and then, more subtly, the climate.

So the responsibility falls upon the shoulders of the modellers. What is the coefficent relating CO_2 concentration to the average temperature on the surface of the Earth? The second is evidently a function of the first, and the function is to a first approximation linear, at least when the processes are slow enough, so there must be a number relating one to another. The role of that number is not to tell us whether or not we should be concerned, but to help us to decide whether we should stay awake at nights. But such a challenge is hopelessly premature: the modellers are still at a loss to know what they should do about real clouds as opposed to the 'average cloudiness'.

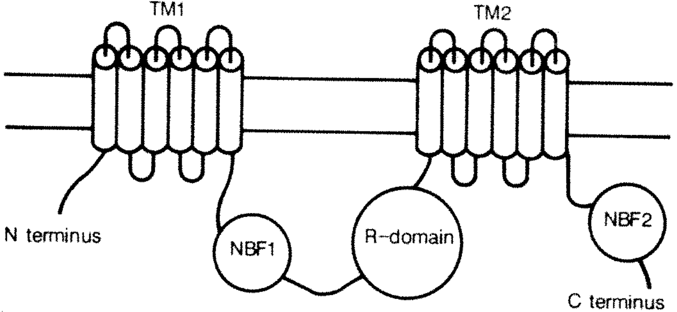
John Maddox

CYSTIC FIBROSIS

A transport problem?

Dagmar Ringe and Gregory A. Petsko

Less than a year ago, the gene responsible for cystic fibrosis, the most prevalent genetic disorder affecting caucasians, was identified and sequenced[1]. As a result, specific sequence changes in genes from affected children can now be correlated with their symptoms. Many of the symptoms of cystic fibrosis are associated with defective transport of chloride ions across epithelia, and Riordan *et al.*[1] initially speculated, among other possibilities, that the gene might encode an epithelial chloride channel. Two papers on pages 362 and 366 of this issue[2,3] reflect recent progress towards a molecular understanding of the disease. Cutting *et al.*[2] have identified four single-point mutations in addition to those already reported in genes from cystic fibrosis patients, and show that all of them cluster in a region of the gene that encodes a putative ATP-binding domain. Hyde *et al.*[3] have attempted to relate genomic defects to altered properties of the protein by proposing a model for this domain, and argue against the hypothesis that the protein functions as a chloride channel.



Schematic model of the cystic fibrosis gene product based on sequence analysis. The segments have been labelled as TM1, TM2 (transmembrane, helical), NBF1, NBF2 (nucleotide-binding domains) and R (unique domain containing the phosphorylation sites). The Phe 508 deletion, the mutation found in 68 per cent of patients, and most of the other mutations observed so far, occur in NBF1. The transmembrane helix mutations occur in TM1.

Cystic fibrosis is an inherited disease characterized by abnormal exocrine secretions, resulting in a devastating variety of symptoms including chronic lung disease. One in every 20 caucasians is a carrier and one in 2,000 live births is affected[4]. The nature of the basic defect leading to the secretory abnormality is unknown.

The gene that is defective in cystic fibrosis encodes a protein of 1,480 amino acid residues (see figure). Hydropathy analysis suggests that the molecule contains 12 transmembrane segments, probably helical, interspersed with three large cytoplasmic domains. The latest reports[2,3] concentrate on two of the cytoplasmic domains, each comprising about 180 amino acid residues. These domains have considerable sequence identity with one another and with similar domains in a superfamily of prokaryotic and eukaryotic transport proteins[5]. The homologous domains contain two consensus sequences that are found in the active sites of proteins that bind and hydrolyse nucleoside triphosphates[6]. The common function of all the previously identified members of the superfamily seems to be the coupling of ATP hydrolysis to the pumping of molecules into or out of the cell.

Cutting *et al.*[2] examined the DNA sequences from exons 9, 10 and 11 and exons 20, 21 and 22 in 38 patients. These exons encode most of the first and second nucleotide-binding domains, respectively. Residue 508 is deleted in the first nucleotide-binding domain in 68 per cent of all patients, but not in these individuals. Four mutations were detected, all in exon 11 in the first nucleotide-binding domain. Three of these mutations cause single amino acid substitutions, and the fourth introduces a stop codon at a position corresponding to Arg 533. Because only a limited region of the gene was analysed, it is not certain that other mutations are present in other exons. Another set of mutations has been reported recently[7]. Patients with these mutations, which occur in the transmembrane regions of the protein, have less severe symptoms. Hyde *et al.* have developed a model for the common nucleotide binding domain in all these ATP-dependent transport proteins[2,8].

The three-dimensional structure of a protein can be predicted on the basis of its sequence if there is a protein of sufficiently similar sequence whose structure is known. Such 'homology modelling' is not possible in the case of the cystic fibrosis product as no structure has been determined for any of the proteins in the superfamily of which it is a member. For this reason, Hyde *et al.* aligned all members of the superfamily and predicted the secondary structure for each sequence. The individual predictions were combined into a consensus secondary structure which showed some similarity to the known pattern of helices and sheets in adenylate kinase. The ATP-binding domain sequences were then folded into a common three-dimensional structure on the basis of the assumed structural similarity with this enzyme.

Given the imprecision of the method used, it is pertinent to ask whether it should be trusted, much less published. The authors admit that the model is likely to be wrong in detail and may even be completely wrong. But it is still a reasonable first attempt and a valuable contribution because it provides a structural model on which experiments can be based, and against which they can be interpreted. Phe 508, for example, is predicted to lie in a loop region with no counterpart in adenylate kinase, so it could lie at an interface between the ATP binding site and the transmembrane regions, where its loss might affect the coupling between ATP hydrolysis and pumping. The amino acid sequence of the 13-residue segment in which all the mutations identified by Cutting *et al.* are found is highly conserved in all members of the superfamily, and the substitutions occur at the three most conserved positions, supporting the assignment of the cystic fibrosis gene product as an ATP-dependent transport protein. In the structural model, these replacements occur in another loop region that is also not found in the adenylate kinase structure, hence the substitutions might not directly affect ATP binding and hydrolysis. But the three missense mutations reported by Cutting *et al.* involve the replacement of small side chains by larger side chains. Site-directed mutagenesis experiments on many proteins have established that such replacements can cause significant alteration in structure if they occur within the hydrophobic core.

Defective transport of chloride ions across epithelia is associated with many of the symptoms of cystic fibrosis[9,10]. But it seems unlikely that the gene product functions as an epithelial chloride channel, despite initial speculations[1]. Hyde *et al.* point out that channels do not require ATP hydrolysis and normally permit the flow of ions in both directions, yet the gene product is homologous with a family of unidirectional active transport proteins. Chloride-channel conductance rates are greater than those of any ATP-dependent transport protein.

Chloride channels appear to differ in different tissues, whereas all cystic fibrosis patients to date seem to have a defect in a single gene. Moreover, the gene product appears too large for an ion channel: its true substrate is likely to be a larger molecule. Finally, ion channels and other transmembrane proteins tend to have characteristic sequence patterns in their intramembrane segments[11]. The pattern of amino acid residues in the 12 putative transmembrane helices does not match the consensus sequences for passive, voltage-gated or ligand-gated ion channels.

Hyde *et al.* argue convincingly that the protein is a transporter but not a chloride channel, but they do not suggest what it transports. It is possible, though, that a function can be proposed based on structural considerations: given the connection between symptoms and the defective regulation of chloride secretion, the protein might transport a molecule that regulates the chloride channel. Mutations could affect chloride secretion by altering the amount of regulator available.

But what is the identity of this molecule? The closest homologues to the cystic fibrosis gene product are the yeast STE6 gene product and the MDR (multi-drug resistance) proteins. Conjugation of hydrophobic drugs to glutathione could be important for *in vivo* export by MDR (ref. 12). Such conjugation has not been observed but the physiological substrates for the MDR proteins have not yet been identified. The substrate for STE6, however, is known to be the farnesyl-derivative of the 12-residue **a**-mating factor peptide[13,14]. The farnesyl moiety is attached by means of a thioether linkage to the C-terminal cysteine of the peptide[15]. From this evidence we can build up a picture of what the substrate molecule for the cystic fibrosis gene product might be like. It would conjugate through a thioether linkage to a glutathione, cysteine or similar residue; it would have a large, hydrophobic organic moiety; and it would have a demonstrable effect on chloride conductance. The products of the arachidonic acid pathway, leukotrienes and prostaglandins, meet these criteria.

Leukotriene LTC4 is known to stimulate mucous and chloride secretion, and has a structure very reminiscent of the farnesylated cysteine of the yeast STE6 substrate. Prostaglandin D2 conjugates with glutathione and cysteine in the presence and absence of the enzyme glutathione S-transferase[16]. Prostaglandin D2 produced the largest change in chloride secretion in experiments with various arachidonic acid metabolites on excised dog tracheal cells[17]. The function *in vivo* of both LTC4 and PDG2 is unclear, but these considerations suggest that one of these molecules (or a similar compound) is the regulator for the epithelial chloride channel and the substrate for the cystic fibrosis gene product. Indeed, an ATP-dependent transporter that pumps glutathione conjugates, including LTC4, from various tissues has already been identified[18]. Inhibition studies imply that this molecule is not identical with the MDR protein[19], but it could still belong to the family that contains MDR, STE6 and the CF gene product. Sequence information about this pump will establish whether the family has yet another member. ☐

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EVOLUTION

Life and times of the guppy

Brian Charlesworth

THE life history of a species or population is described by such variables as the age of first reproduction, and the mortality rate and reproductive success of individuals as functions of age. Life histories vary greatly between taxonomic groups: for example life spans in eutherian mammals range from a few months in small insectivores to 80 years or more in humans[1]. The age of reproductive maturity and number of offspring per litter show a similar diversity[2]. A substantial body of theory has grown up that endeavours to make predictions about the ways in which natural selection moulds life history, and to explain the diversity of life-history patterns[3,4]. A new study on page 357 of this issue[5] puts the standard explanation of life-history differences between natural populations to the test. It is the first case in which life-history differences observed in nature have been reproduced in the laboratory.

Life-history variation between populations of the guppy (*Poecilia reticulata*) are thought to reflect differences in the main species of local predators. In some places, predators take large, adult guppies, but in others the juveniles bear the brunt. Guppies from the former class of population mature earlier, and females allocate a greater proportion of their body mass to the reproductive system and have a larger number of offspring per brood compared with guppies from the second type of population[5]. These differences accord with the predictions of the standard 'reproductive effort' model of life-history evolution[3,4,6]. In species with multiple episodes of reproduction, this model assumes that, for individuals of a given age, the allocation of resources between reproduction and other activities (such as survival to the next time of reproduction) may vary.

Other things being equal, the greater the reproductive effort of an individual (that is, the larger the proportion of resources allocated to reproduction), the lower its chance of survival. The life history that is evolutionarily optimal can be calculated for this model[3,4,6]. One prediction is that the level of reproductive effort favoured by natural selection should be strongly influenced by the relative amounts of mortality of adults and juveniles, imposed by external factors such as predation. Relatively high adult mortality favours the evolution of a life history in which there is high reproductive effort. The intuitive reason for this is clear: if the chance of getting through to the next opportunity for reproduction is inherently low, one might as well reproduce now, rather than get killed off. In addition, relatively high mortality tends to select for genes that increase survival and reproduction early rather than late in life[3].

Differences between taxa can often be interpreted in terms of this demographic influence on life-history evolution[3]. For example, there is a positive correlation among bird species between survival rates in the wild and the age of reproductive maturity, and a negative correlation between clutch size and survival[7]. But comparative studies are always open to the objection that factors other than those imagined in one's favourite theory are responsible for the correlations observed. This objection is met in various studies in which artificial changes in the demography of laboratory populations produce evolutionary responses of the kind predicted by life-history theory[8,9]. Of course, such studies merely show that genetic variability of the kind postulated in the models can be exploited by selection: they do not prove that the invoked selective agents are actually responsible for producing the observed differences.

Reznick *et al.*[5] have used the guppy system in a neat study that combines both comparative and experimental approaches. In 1976, they transplanted a population of 200 individuals from a river in Trinidad with high predation of adults to a tributary where guppies were previously absent, and the local predator was a species that ate juveniles rather than adults. The transplanted population has flourished, and has been monitored at intervals between 1978 and 1988 — a total of 11 years (30–60 generations) in the new environment. Females in the new site now tend to produce smaller brood sizes and have lower reproductive effort than those from the original locality. Laboratory-reared guppies from both localities showed a broadly similar pattern of differences, indicating that there has been a genuine evolutionary response to the changed conditions. An interesting aspect of the laboratory data is that only reproductive effort early in life seems to have been reduced in the introduction site. This is similar to the results obtained for *Drosophila* populations selected for longevity[8], and presumably reflects the greater selective impact of changes in early life that is at the core of evolutionary explanations of ageing[3,10]. It would be interesting to know whether age-specific mortality of the guppies under laboratory conditions has also been modified. ☐

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CHEMISTRY

A new twist to self-assembly

Edwin C. Constable

ATTEMPTS by synthetic chemists to imitate the natural world have flourished in the past decade, which has seen the design of artificial enzymes, photosynthetic species and self-assembling molecules. On page 339 of this issue[1], Lehn *et al.* describe the preparation of a new class of compounds that mimic the double-helical structure of DNA. One of the remarkable features of these compounds is the spontaneous assembly of the double-helical arrays from linear precursors through their interaction with monovalent copper centres. Their structure is a curious 'inside-out' analogue of that of DNA.

Copper(I) has a preference for near-tetrahedral geometry, and the secret of helix-building in the work of Lehn's group lies in the choice of a polynucleating ligand that can bind in a bidentate mode at a sequence of metal centres. Ligands based on oligopyridines prove to be ideal for this purpose, and the tetrahedral geometry at each centre combined with the correct 'spacer' groups between ligands allows the construction of double-helical ligand arrays about a series of metal centres[2] (Fig. 1).

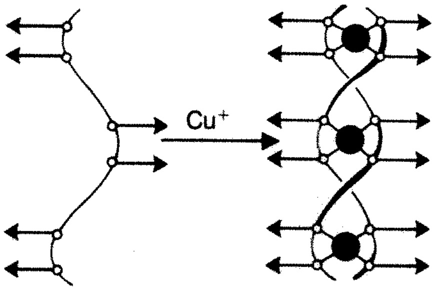

FIG. 1 Lehn's method for the spontaneous assembly of double helices. Key (both figures): nitrogen donor atom, ○; reactive functional group, •; copper, ●; nucleoside, ⟶.

In their new work, Lehn's group have introduced deoxynucleoside substituents onto the outside of double helices constructed about three or five copper(I) centres. The molecules so constructed show many of the characteristic structural features of nucleic acids, but the overall structural consequences are very different. Lehn's new compounds and DNA all have double-helical geometries, contain deoxynucleosides, are charged and exhibit stacking interactions between approximately coplanar aromatic rings. But in DNA the deoxynucleosides are within the double helix, the stacking is between the deoxynucleosides, and the overall charge is negative; whereas in Lehn's compounds the deoxynucleosides are on the outside of the double helix, the stacking is between the oligopyridines and the overall charge is positive. As Lehn *et al.* point out, the structures of the new molecules are strikingly reminiscent of Linus Pauling's original proposal for the structure of DNA, which had the deoxynucleosides on the outside and the charged phosphate groups facing in.

These interesting new compounds offer opportunities to investigate an entirely new class of DNA–molecule interactions. The extra-helical deoxynucleosides of Lehn's compounds might intercalate or enter into triple-helix hydrogen-bonding interactions with DNA. The metal complexes are positively charged and should show strong electrostatic interactions with the negatively charged surface of DNA and possibly a helix–helix recognition for the major groove. The development of double-helical complexes containing other photoactive metal centres might allow the formation of new site-specific nucleases of the type investigated by Barton *et al.*[3]. Those octahedral transition-metal complexes containing substituted 1,10-phenanthroline ligands exhibit site-specific non-covalent interactions with DNA. If the metal centre is photo- or redox-active, it is possible to couple photochemical or oxidation changes at the metal centre to oxidative cleavage reactions of the sugar-phosphate backbone[4].

The specific geometrical requirements of metal centres in their coordination compounds has been exploited in a number of other elegant examples of molecular architecture. Sauvage and his group have made extensive use of the tetrahedral copper(I) centre in assembling a series of particularly beautiful molecules. The simplest of these are the catenanes, which may be viewed as two (or more) links from a molecular chain. These are assembled by ring closure of two functionalized bidentate 1,10-phenanthrolines coordinated to a tetrahedral copper(I) centre[5] (Fig. 2*a*).

If two of these 1,10-phenanthroline derivates are linked by a suitable spacer chain, a double-helical complex is assembled in a process similar to that observed by Lehn *et al.* Ring closure in this compound leads not to a catenane but to a coordinated trefoil knot, from which the free ligand may be obtained upon treatment with cyanide[6] (Fig. 2*b*). This remarkable synthesis could be achieved only by using metal-ion control over the conformation of the coordinated reactants.

Terdentate ligands may also be used for the spontaneous assembly of double-helical arrays, an example having been reported recently by Williams's group[7] (Fig. 2*c*). Once again, copper(I) was used as the metal centre to control the helix formation. Very recently, second-row transition metals have also been shown to direct the spontaneous assembly of double-helical ligand arrays[8].

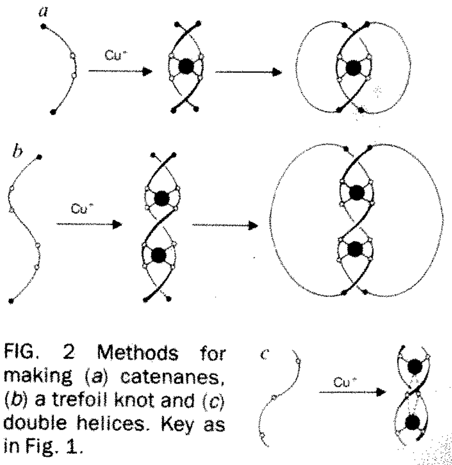

FIG. 2 Methods for making (*a*) catenanes, (*b*) a trefoil knot and (*c*) double helices. Key as in Fig. 1.

Although many of these examples of topological control rely on a metal ion to control the geometry of reactants, it is possible to do this using other intermolecular interactions. Stoddart *et al.*[9] have recently made use of stacking interactions between electron-rich and electron-poor aromatic systems in a high-yield, metal-free, one-pot synthesis of catenanes (see also ref. 10).

The ability of intermolecular interactions, particularly those involving metal ions, to control the self-assembly of molecular aggregates is an area of intense current interest. The use of such interactions allows very specific control over the conformation and stoichiometry of the products. The methodologies emerging from these studies represent an amalgam of 'organic' and 'inorganic' techniques and expertise, and offer powerful new approaches to the synthesis of compounds that are of interest for their glorious shapes as well as their potential to exhibit novel and specific interactions with biological molecules. ☐

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TRANSGENIC MICE

Gene expression and hair-loss

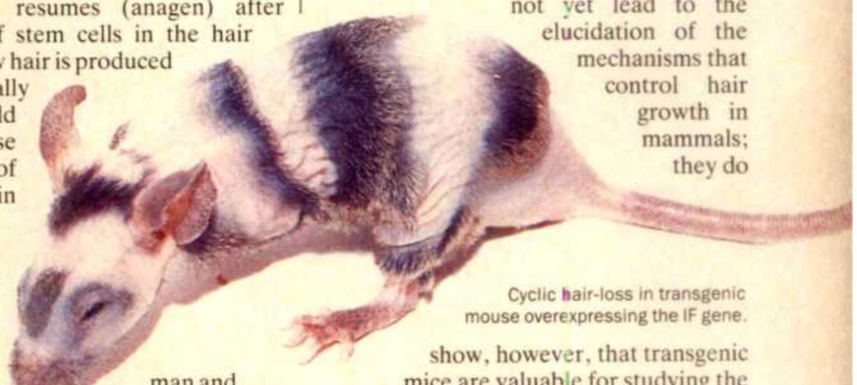
Paul E. Bowden

THE regulation of gene expression and the biological consequences of gene manipulation can be studied by the stable incorporation of 'foreign' genes either into somatic cells, or into the germ line of recipient organisms. Transgenic mice result from the successful integration of foreign genes into the germ line; in this way, the introduced genes are subject to the complete developmental programme of the organism. The transgenic approach has recently been used to study the expression and regulation of keratin genes, which encode the structural proteins of hair and skin. In a new report, Powell and Rogers[1] describe the dramatic consequences of expressing a single component of sheep hair (a type II intermediate filament (IF) keratin protein) at high concentrations in transgenic mice. Although expression of this keratin IF gene is tissue specific, its overexpression leads to an imbalance of the normal ordered array of hair structural proteins. The result is a weakened structure, and breaking of the hair shaft (wool fibre) leads to premature loss of hair (see figure). This does not seem to be simply a consequence of expressing a sheep keratin IF gene in a mouse, as mice having a lower level of transgene expression (50 copies or less) suffer no hair-loss. Furthermore, the amino acid sequences of the keratins of sheep wool and mouse hair are very similar[2].

Hair from all mammals possesses a common structural plan and undergoes a cyclic programme of growth and differentiation. The hair follicle is first formed during embryonic development by downgrowth and specialization of the epidermal basal cells, which also differentiate to form the outer layers of the skin[3]. A complex multicellular structure is formed, which after a period of hair-specific differentiation and growth, produces a hair that protrudes beyond the surface of the skin. The follicle then attains a dormant state (telogen) during which the fully formed hair is retained in the follicle. Hair growth then resumes (anagen) after stimulation of stem cells in the hair bulb and a new hair is produced which eventually displaces the old one. Because the activity of hair follicles in the mouse is synchronized, waves of new hair growth occur in regular cycles[4], whereas in man and sheep, a mosaic pattern of active hair follicles interspersed with dormant follicles is seen. Thus, the normal hair cycle, characterized by periods of active growth followed by dormancy, produces a continuous refurbishment of the coat (pelage) without significant loss of hair in any specific area of the skin.

The hair fibre is composed of a cylindrical cortex with a central core (medulla) surrounded by a cuticle. The hair cortical cells contain a regular and distinctive arrangement of filaments (keratin IF proteins) embedded in a matrix of cysteine-rich and glycine–tyrosine-rich filament-associated proteins[5]. Between eight and ten hair-specific keratin IF proteins have been described[6,7] in addition to the keratin IF proteins expressed in other epithelia[8,9]. But the synthesis of filament-associated proteins seems to be a late event in hair differentiation and follows the synthesis of keratin IF proteins[10,11]. So precise regulation of the sequential expression of at least 20 genes is required to construct the filament–matrix array of the hair cortical cell.

Powell and Rogers[1] demonstrate that in transgenic mice with many copies (roughly 250) of the sheep wool keratin IF gene, the overproduction of keratin filaments leads to a reduced synthesis of the sulphur-rich and glycine–tyrosine-rich filament-associated proteins. This disrupts the normal ratio of filament to filament-associated protein, which has profound effects on hair structure and strength. The hairs produced are wavy and tend to break just below the skin surface. Similar findings have been described in several disorders of human hair[12]. This leads to the premature loss of hair that the authors observed in their transgenic mice. The new experiments do not yet lead to the elucidation of the mechanisms that control hair growth in mammals; they do show, however, that transgenic mice are valuable for studying the expression and regulation of the many structural genes involved in this complex system, and that the biological consequences of manipulating the genome can be difficult to predict. ☐



Cyclic hair-loss in transgenic mouse overexpressing the IF gene.

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ASTROPHYSICS

Stellar birth control

A. P. Whitworth

STAR formation is concentrated in giant molecular clouds, which range in size from 10 to 100 parsecs, and in mass from 10^4 to 10^7 solar masses. The molecular clouds are very fragmented and porous, with most of their mass concentrated in numerous dense clumps. The challenge facing the astrophysicists attending a recent meeting* was to establish the details of this structure, how it is created, and how it evolves. For these are the processes that control the efficiency of star formation in a giant molecular cloud, the initial mass function (the relative proportions of stars formed with different masses) and the initial orbits of binary stars and star clusters — in short, the processes of stellar birth control.

Evidence of the porosity of giant molecular clouds (GMCs) comes from observations of emission lines from ionized carbon, atomic carbon and carbon monoxide in the cloud next to the nebula M17 (J. Stutski, Max-Planck-Institut, Munich). The coincidence of the peaks in the emission lines from these different species implies that far-ultraviolet radiation penetrates deep into the cloud, and thus maintains (by photodissociation and photo-ionization) a distribution of atomic and ionized carbon that is broadly coincident with the distribution of carbon monoxide. This radiation travels between patches of CO with little attenuation, ensuring that each patch is surrounded by an envelope of the atomic and ionized species.

The sub-structure of GMCs is fractal (E. Falgarone, IRAM Grenoble). In maps of molecular line emission, the isophotes (lines of constant brightness) satisfy the relationship $P \propto A^{0.68}$, where P is the perimeter of the isophote and A is the area enclosed. The amount by which the exponent exceeds ½ (the value expected for isophotes with simple shapes) is a measure of the 'knobbliness' of GMC sub-structure. The fact that this relation holds over a large range of P (from 0.02 to 3,000 parsecs) indicates that the same degree of knobbliness is found on widely different scales. Falgarone's result is remarkable because the fractal dimension is the same as that observed in two-dimensional slices through high-Reynolds-number (well developed) turbulence in the laboratory, and therefore seems to corroborate the theory that GMCs are turbulent.

It is surprising that interstellar turbulence mimics terrestrial turbulence so closely. Terrestrial turbulence is essentially incompressible and not self-gravitating; turbulent energy is injected continuously on the largest scale and cascades without dissipation through a large inertial range until being dissipated eventually on much smaller scales. Interstellar turbulence, on the other hand, is compressible and self-gravitating; turbulent energy is injected and dissipated stochastically and on a wide range of scales, perhaps even being amplified gravitationally in a cascade to larger scales.

The Cone Nebula, in the constellation Monocerus, is the prototypical example of a cometary nebula. The bright 'fireball' at its apex is a cloud of ionized gas which casts a shadow in its wake.

Two sources of turbulent energy in GMCs are ionizing radiation and stellar winds from associations of massive, luminous OB stars. In the Orion/Ophiuchus star-forming region, the influence of the central OB association is felt out to a distance of 80 parsecs (J. Bally, AT&T Bell Laboratories), as indicated by the systematic orientation of cometary nebulae. These nebulae are so called because of their comet-like shape, characterized by a very dense head and a less dense tail, the whole of which may be enclosed in a sheath of ionized gas. The Cone Nebula (see figure) is prototypical. In Orion/Ophiuchus the heads all point towards the central OB association, suggesting that the nebula boundary is defined by a bow shock and/or ionization front where the collective stellar wind and/or ionizing flux from the central stars encounters the material in the head. The tail is simply the shielded region in its wake.

Cometary nebulae are important sites of star formation. The heads sustain a high formation rate, resulting in populous clusters which include high-mass stars; whereas the tails support only a slow rate of sparsely distributed, predominantly low-mass, star formation (P. Myers, Harvard Center for Astrophysics). The masses of protostellar cores reflect the properties of the clouds within which they form, in the sense that more massive cores (and hence presumably more massive stars) form in more massive clouds and hence in deeper, steeper background gravitational potential wells. If this tendency is universal, it explains why star formation in cooling flows (extended envelops of very hot, X-ray-emitting gas around elliptical galaxies) produces only low-mass stars, and it suggests that globular clusters had a high proportion of massive stars early in their evolution.

One can speculate that 10–20 million years after its formation the head of a cometary nebula will be dispersed by the ionizing radiation and winds from the massive stars which have formed within it. The tail will probably persist as an isolated filament of the type observed in Taurus (Y. Fukui, Nagoya).

Despite the wealth of observational data on star-forming regions, the connection between observation and star-formation theory remains tenuous. In star-formation theory, there is only one certainty: stars condense out of interstellar gas through the effect of self-gravity. There is also a near-certainty: self-gravity first pulls together the material to form a star-cluster and then from this it forms the individual stars. But as yet, there is no observational confirmation of either the certainty or the near-certainty. We do not see protostars or protoclusters contracting under self-gravity. On the large scale, those parts of star-forming regions that reveal themselves to us appear tenuous and filamentary, more akin to the wispy cloud patterns of a summer's sky than to massive bodies bent on collapse.

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* *Fragmentation of Molecular Clouds and Star Formation*, IAU Symposium No. 147, Grenoble, 11–15 June 1990.

On the small scale, we see cold, self-gravitating molecular clumps and infrared sources which indicate where stars have formed or are in the final stages of formation; but there is no unique signature of inward motion.

The observations indicate that thermal instabilities are at least as important as self-gravity in generating sub-structure within GMCs. Numerical simulations (J. Chiéze, CEA Bruyeres) show that when the ambient ultraviolet radiation field is reduced by one magnitude of extinction through the eclipse of a nearby hot star by a cloud (perhaps a cometary head), cooling of the shadowed region may cause its density to increase by two orders of magnitude in a fraction of a million years. This seems to provide a very promising mechanism for generating cometary tails and filaments □

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PALAEOCLIMATE

Puzzles from the tropics

David Rind

THE reconstruction of changes in temperature and precipitation in equatorial Africa over the past 40,000 years, reported by Bonnefille *et al.*[1] on page 347 of this issue, addresses a problem of prime importance for our ability to predict future changes to the global climate.

Why is this so? In assessments of the anthropogenic influence on global climate in the coming century, the response of temperatures in the tropical regions represents perhaps the greatest uncertainty. The general circulation models (GCMs) used by different groups in estimating the degree of global warming to be expected from increased levels of atmosphere CO_2 give results for the tropics that differ by a factor of two (ref. 2). These differences affect the predicted change in latitudinal temperature gradients, and thus in storm tracks and intensities, in rainfall patterns — indeed, in the whole general circulation[2]. More specifically, if the tropics warm significantly, there is the likelihood of a substantial increase in droughts[3].

Although the reasons for the differences in the models' tropical response are not yet clear, they may be associated with the different convection or radiation schemes used. To decide which of the predicted responses is the most likely, researchers have turned to the palaeorecord — only to find that here the discrepancy is just as large. The 1981 CLIMAP[4] data set indicated that, at the peak of the last ice age, tropical sea surface temperatures were relatively warm. This implies a low tropical sensitivity to severe climate perturbation. But the land-based glacial and pollen data indicate that the last ice age saw snowlines and vegetation zones lowered by about 1 km in mountains at a wide range of tropical and semi-tropical locations[5]. Assuming that this depression corresponds to a depression in temperature, and given a temperature drop with altitude of 5–6 °C km^{-1}, this would imply substantial tropical cooling. GCM results show that these land and sea temperature reconstructions are not consistent[5], leaving considerable uncertainty about what the tropical climate was really like during the last ice age.

Looking further back in time, one finds that the record becomes even murkier. In the warm climate of the Mesozoic, the tropics seem to have been substantially warmer[6], but during periods in the Cenozoic that were apparently very warm at high latitudes, there is little evidence of any warming in the tropics[7]. It is fair to say that until now the record has not helped us to understand how the tropics are likely to change in the future.

Bonnefille *et al.* address this issue by attempting to reconstruct the climate record for the Last Glacial Maximum at an intermediate altitude of about 2 km. This is an important difference from the glacial and pollen records that have been reported previously[5], which have generally come from higher elevations. Furthermore, instead of simply using temperature lapse rates (variation of temperature with altitude) to generate a cooling estimate, they use modern analogues to relate the vegetational changes recorded in the pollen data to temperature and precipitation changes. This allows them to separate the effects of changes in temperature and precipitation on ecosystem distributions. They conclude that temperature changes derived from lapse-rate calculations using the pollen record overestimate tropical cooling by ignoring the fact that part of the pollen changes are induced by precipitation. Thus tropical cooling should be less than has been assumed, implying a lower tropical climate sensititivity.

TEMPERATURE CHANGE FOR THE LAST GLACIAL MAXIMUM IN THE GISS GCM FOR EQUATORIAL AFRICA

Altitude	△*T* (CLIMAP SSTs)	△T (CLIMAP – 2 °C)
Sea surface	–1.3°	–3.3°
2 km	–2.0°	–4.0°
4 km	–2.4°	–4.9°
6 km	–2.9°	–6.0°

Sea surface temperature changes are zonal averages for 4° S.

Does this result settle the issue? Unfortunately not, although it is an important piece of evidence. Bonnefille *et al.*'s reduced cooling estimate for the Last Glacial Maximum, 4 ± 2 °C, is not sufficiently different from the lapse-rate estimate of 5–6 °C to be definitive, especially considering the uncertainties inherent in the authors' time-lagged approach. More importantly, as the authors note, they are considering a lower elevation than most other researchers. In the drier climate of the ice-age tropics, the atmospheric lapse rate would be expected to increase (to move somewhat closer to the dry adiabatic value of 10 °C km^{-1} rather than the moist adiabatic value of 5 °C km^{-1}); this means that temperature changes should be somewhat smaller at lower elevations. A 4 °C cooling at 2 km is perfectly consistent with a 5 °C cooling at 4 km, and neither can be obtained at low latitudes from a GCM using the warm CLIMAP sea surface temperatures. So the results of Bonnefille *et al.* are actually quite consistent with greater tropical sensitivity. This is shown in the table, which presents the temperature changes as a function of altitude produced by the Goddard Institute for Space Studies GCM in the grid box that encompasses the tropical African observation point, for two sets of conditions: using the glacial sea surface temperatures deduced by CLIMAP and using those temperatures reduced by 2 °C. In both cases temperature depressions are larger at greater elevations; for the lower sea surface temperatures, the effect becomes larger because the atmosphere becomes drier and so the lapse rate increases.

This lapse-rate argument cannot be carried too far, however. For instance, one cannot assume that the tropics dried out sufficiently to increase the lapse rate until the warm sea surface temperatures become consistent with the substantial cooling aloft. The movement of glaciers down mountains is more difficult in a drier climate, and so requires a still greater temperature depression[5]. But lower temperatures would then mean an even greater lapse rate from high altitudes down to the warm surface, which would imply even drier conditions . . . and the whole argument regresses until there is no moisture left with which to form glaciers. For the same reason, it is unlikely that precipitation changes have caused most researchers to overestimate the pollen-derived cooling in other locations: the regions of glacial and vegetation-line descent are often contiguous, and the two phenomena have the same temperature requirement (cooling) but opposite moisture requirements. In principle, the use of both data sets should allow the changes in temperature and moisture to be bounded.

How are we to solve this problem? We definitely need more studies like that of Bonnefille *et al.*, exploring tropical and semi-tropical palaeo-temperatures at a range of elevations and at widely separated locations. From the modelling end, we need to determine why the different GCMs produce different tropical responses to increases in CO_2. The uncertainty in tropical climate sensitivity emphasizes that we do not yet understand global climate sensitivity, and makes dynamical and regional predictions for the coming century that much more difficult. □

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CELL BIOLOGY

Trawling for receptors

Graham Warren

ENDOCYTOSIS is the process whereby substances on cell surfaces are enclosed in membrane-bound vesicles and taken into the cell. These substances then pass through the cell in what is usually thought of as an orderly itinerary of discrete stages (Fig. 1). But video images of living cells show that the endocytic pathway may be more interconnected than was previously thought. In the conventional picture, receptors that cluster in clathrin-coated pits in the plasma membrane pass through a series of internal, membrane-bound compartments[1], the first of which is the early endosome. Transferrin receptors are recycled almost immediately from this compartment back to the cell surface[2] whereas receptors for epidermal growth factor pass to late endosomes. In the process, these receptors become incorporated into budding vesicles that pinch off and congregate within what can then be called a multi-vesicular body[3] (Fig. 2). These are processed further[4] and are eventually delivered to lysosomes. There is general agreement that each compartment in the endocytic pathway is a discrete, separate entity. In fact, it is difficult to see how parts of the pathway could function were this not the case. But on page 335 of this issue[5], Hopkins *et al.* report observations that strongly suggest that the early part of the endocytic pathway is a network of interconnected tubules.

What Hopkins *et al.* have done is to use different fluorescent tags to follow both the transferrin and epidermal growth factor (EGF) receptors in living cells using video recording at low light levels. At steady state, the transferrin receptor reveals an interconnected, tubular network, through which occasional pulses of receptor appear to be moving at high speed (up to $1\ \mu m\ s^{-1}$). The network is very extensive but has not been seen before because it is labile: sensitive to low levels of ultraviolet light, lowered temperature and the chemical fixatives that are normally used to prepare samples for immunofluorescence and electron microscopy.

The effect of adding EGF is particularly dramatic as anyone who has seen the video will confirm. The network grows and the EGF receptors appear rapidly in boluses that move along the tubes at speeds of around $0.05\ \mu m\ s^{-1}$. Electron microscopy confirms that these boluses are multi-vesicular bodies (MVBs). The MVB has always been thought of as a discrete organelle and any tubular extensions have been short and thought to be

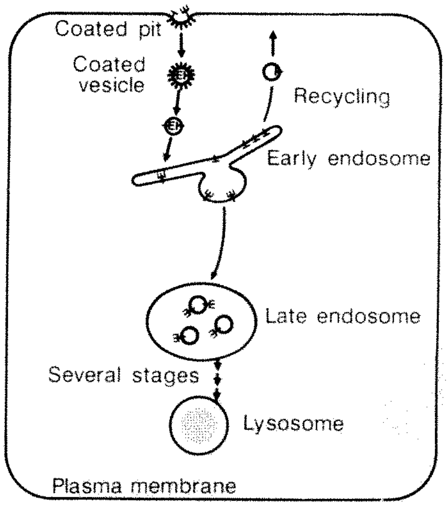

FIG. 1 The usual picture of receptor-mediated endocytosis. EGF receptors (—E) and transferrin receptors (T) are ingested by the cell and pass through a series of stages before being respectively destroyed or recycled. Multi-vesicular bodies can form at almost any stage from maturing endosomes or early lysosomes.

Ann Bishop (1899–1990)

THE death on 7 May of Ann Bishop marks the end of an era during which the direction of British protozoology was influenced by three remarkable women — Doris Mackinnon (1887–1956), Muriel Robertson (1883–1973) and Ann Bishop herself.

A Manchester zoologist by training, Bishop worked all her life in Cambridge and made numerous and diverse contributions to protozoology including the nuclear and ciliary structural biology of the ciliate *Spirostomum* and various aspects of the biology of *Trichomonas* and *Entamoeba* species. She was involved in the discovery that the causative agent of blackhead in turkeys was *Histomonas meleagridis*, but it was as a malariologist that she became best known.

In the years overlapping the Second World War, Bishop made vitally important contributions to the understanding of drug resistance in malaria. In the late 1930s, as new antimalarial drugs were becoming available, there was great concern that these might easily evoke resistance as had happened with antibiotics. But there were few procedures or protocols that had been laid down for the investigation of this problem, and Bishop had to develop experimental systems to assess resistance. Her choice was *Plasmodium gallinaceum* in chickens, and by 1938 she had demonstrated resistance to proguanil. She also discovered the time at which resistance occurred, that it persisted even after mosquito transmission and, more importantly, that there was cross-resistance between proguanil and other dihydrofolate reductase inhibitors which she later discovered extended to pyrimethamine. A decade later, with the availability of rodent malarias, she turned her attention to these and established procedures, which have now become standard in laboratories all over the world. Bishop also set herself the task of discovering how drug resistance occurs, a problem that remains unsolved. She also began to investigate the trigger for gametocytogenesis in malaria parasites, a question that 25 years later is still unanswered.

From 1942 to 1964, she was director of the Medical Research Council's Chemotherapy Research Unit at the Molteno Institute in Cambridge where many British and foreign scientists trained in experimental parasitology and benefited from her wide experience. Her work was recognized by her being elected to a Fellow of the Royal Society in 1959, a rare accomplishment for a woman at that time, especially for one engaged in essentially medical research. Those who knew Ann Bishop will remember her with affection as a great scientist and a gracious lady. F. E. G. Cox

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involved in removing the last of the recycling receptors[6,7]. If it is not discrete but part of an extensive endocytic network through which it moves, then one's view of this organelle must change. One could instead think of it as a trawler that scours the tubular network for receptors, allowing transferrin receptors to pass through the net but trapping and internalizing a cargo of EGF receptors (Fig. 2). Movement of these MVBs through the network might be facilitated by a microtubule-based system[8] and the net could be provided by whatever it is that makes up the coat responsible for budding into the MVBs.

Exciting though these observations are, they do pose many problems. The most obvious are those that are easily solved by discrete compartments but not so easily by networks. As an example, movement along the endocytic pathway is characterized by a fall in pH (ref. 9) which is easily understood if each compartment is discrete and has different proton-pumping capabilities[10,11]. In a network, one would need to place strong proton pumps in the membranes of the MVBs and sinks elsewhere, otherwise the pH would equilibrate rapidly throughout the network. It may even be that the network is not stable with time, but fragments and fuses in a manner similar to that of the endoplasmic reticulum[12]. Those fragments containing the most proton pumps could then reach a lower pH than would be possible as part of a network.

It is also difficult to reconcile these observations with results obtained in other systems. To take just one example, using a polarized cell line, there is good evidence to show that the contents of early and late endosomes do not mix[13], which either means that the cells have different endocytic pathways or that the terms 'early' and 'late' as applied to endosomes have different meanings in the two systems. This highlights one of the chief problems in the endocytic field — the absence of good markers for the early endocytic pathway. Until such markers are available one can only compare results generated using different methods within one cell system. Meanwhile, the observations reported by Hopkins *et al.* provide a new view of the endocytic pathway and any future models will have to take these observations into account. ☐

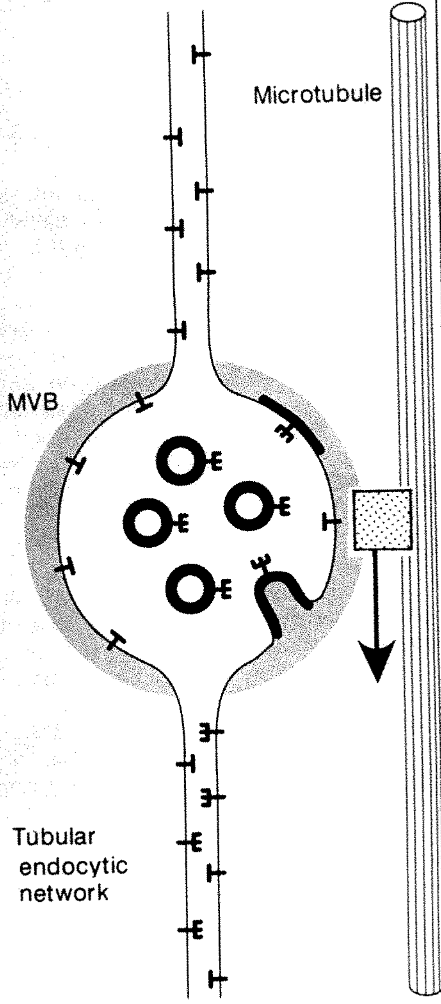

FIG. 2 Multi-vesicular bodies (MVBs) appear to be continuous with a tubular endocytic network and to move through these tubules using microtubule-dependent motors (arrow). As the MVBs move, one could imagine that EGF receptors (—E), but not transferrin receptors (T), would become incorporated into budding vesicles that pinch off and congregate within the lumen.

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ACTIVE GALACTIC NUCLEI

A long, deep look at the shape of Seyfert galaxies

Julian H. Krolik

ACTIVE galactic nuclei are distinguished from normal galaxies principally by their tremendous power output from only a tiny volume of space. Another distinctive feature is the presence in their spectra of very strong and broad emission lines. The results, reported at a recent meeting*, of a worldwide campaign to monitor variations both in the emission-line and the ultraviolet continuum fluxes of a particular active galaxy, the Seyfert galaxy NGC5548 (refs 1–3), provide our first detailed picture of the density and physical state of the line-emitting gas in this type of object[4].

The emission lines from active galactic nuclei are at once both mundane — in the sense that they are radiated by such common atoms and ions as H, C^{2+}, C^{3+}, N^{4+} and Mg^{+} — and dramatic, often carrying 10 per cent of the total power from the galactic nucleus and implying (from their Doppler shifts) gas velocities of up to 10^4 km s^{-1}. By the mid 1970s, it was generally agreed that the lines derive their power via photo-ionization by the ultraviolet and X-ray continuum emission; and by the early 1980s there was also general agreement about the kind of physical conditions that exist in the line-emission region. Combining the latter inferences with a measurement of the total ionizing power of the active nucleus, it is possible to estimate the distance between the continuum source and the line-emitting material. Direct experimental tests of this picture were lacking, however, as were data on the internal structure of line-emitting regions. The conference bore the happy news that both these problems have now been overcome.

The basic idea[5,6] behind the work reported is that if the emission lines are powered by the continuum radiation, fluctuations in the continuum drive corresponding fluctuations in the local line emissivity within the emission-line region. The time required for a local readjustment of the ionization state and temperature following a change in the continuum is thought to be less than one hour, whereas the light travel time across the line-emission region is expected to be in the range from days to years, so fluctuations in the line flux should mimic delayed and smeared versions of the continuum fluctuations. If the fluctuations remain small enough to drive line emission linearly, the line light curve is related to the continuum light curve by a simple convolution with a response function. Deconvolution of the two light curves for the response function would then produce a one-dimensional map of 'marginal line emissivity' (the partial derivative of the line flux with respect to the continuum flux, averaged over each paraboloidal surface of constant delay). Unfortunately this requires many observations to describe adequately the light curves, and the interval between observations must be chosen carefully to match the relevant timescales.

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* Conference on *Variability in Active Galactic Nuclei*. Georgia State University, 2–4 May 1990.

Such careful sampling is difficult to achieve in ground-based astronomy because observing is subject to uncontrollable interruptions, of which obscuration by the Moon's light and by bad weather are the worst examples. But orbiting satellites are subject to neither of these constraints, and so in 1987 a campaign was organized to use the International Ultraviolet Explorer satellite to observe the Seyfert galaxy NGC5548 every four days over a span of eight months. From this collection of spectra, time series for three continuum bands and eight emission lines were compiled. At the same time, a large-scale complementary ground-based campaign was mounted to gather data (albeit with less regular sampling) on the behaviour of the optical continuum and emission lines.

Ionization conditions

With these data in hand it has become feasible to attempt the deconvolution. The result is that (at least in this example) the lines fall neatly into two groups: those radiated in relatively high-ionization conditions (H II, N V, C IV, Si IV and Lyman α) and those from lower-ionization conditions (C III], Mg II, O I and Hβ). The first set responds most strongly to changes in the continuum at zero lag, the response tapering off almost to zero after 20 days. The second set responds after a considerably greater delay, but only for the C III] and Hβ (ref. 7) lines was the signal-to-noise ratio high enough to permit a good look at the response function: for both of these lines, the response at zero delay is quite weak, builds to a peak after 20 to 30 days, and falls steeply at larger lags.

Such one-dimensional maps are interesting, but they are less than satisfactory because they give the marginal emissivity only as a function of delay along our line of sight. Whatever symmetry truly characterizes the emission-line region, it is very unlikely to be defined by our line of sight. The shapes of the response functions, however, give a good guide to the true symmetry. When the lines are radiated isotropically (a good approximation for many of those measured), response functions peaking at zero lag must be associated with quasi-spherical regions, whereas the simplest symmetry for those peaking at larger lags is cylindrical. Thus it seems that (at least in NGC5548) the emission-line region has two sub-regions: an inner, roughly spherical zone responsible for the high-ionization lines and an outer, flattened zone responsible for the low-ionization lines.

The fact that five response functions are obtained from the same (high-ionization) region permits a still more detailed analysis. At each lag τ, the overall amplitude of the high-ionization response functions reveals the solid angle subtended by the highly ionized material on a sphere of diameter $c\tau$; the ratios of these functions reveal (with the help of some atomic-physics calculations) the physical conditions in the gas. Adding into the analysis the mean ionizing power, a new estimate may be made of the distance from the continuum to the line-emitting region; satisfyingly, this distance corresponds to the radius of the sphere ($c\tau/2$) to within a factor of 2–4. Such self-consistency adds considerable confidence to the inferences drawn about physical conditions: the high-ionization line-emitting material in NGC5548 is spread over spherical shells ranging in radius from 4 to 14 light-days, with the greatest amount of line radiation coming from the shell with a radius of about 8 light-days; the pressure stays fairly constant throughout this region, maintaining a density of around 10^{11} H atoms cm^{-3}; and the thickness of individual line-emitting clumps is less than about 10^{11} cm.

Because only two lines are available to constrain the models, the structure of the outer (low-ionization) region is determined less well. Although it must be flattened cylindrically, the response functions could correspond equally well to a ring with a radius of about 25 light-days lying face-on to our line of sight, or a ring with a radius of about 100 light-days viewed almost edge-on.

These studies are producing a higher level of understanding of active-galaxy emission lines. Both direct confirmation of inferences from models and detailed structural information are now accessible, provided that the necessary large-scale coordinated efforts can be mounted. Judging by the number of new monitoring programmes being promoted at the meeting, that is a cost that will soon be met. ☐

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DAEDALUS

Equilibrated junk

What should we do with our rubbish? Dumping it as landfill is increasingly unacceptable, clear-air lobbyists oppose incineration even at sea, and few citizens are dedicated enough to sort and classify the stuff for recycling. Daedalus recalls composting, that ancient method of reducing organic waste to a uniform and fertilizing humus. He is now adapting the technique to urban rubbish.

His idea is simply to heat the rubbish to such a temperature that all possible chemical reactions between its components go to completion. The end product, DREADCO's 'Rumus', would be the stablest, lowest-energy compound or mixture of compounds that could be formed from the initial composition. The concept will be familiar to those astrochemists who calculate what minerals would preferentially separate from any assumed primaeval planetary material as it cooled. Rubbish presents a harder challenge. The only safe bets are that Rumus will be extensively chemically bonded, and will therefore be a solid; and since it has the lowest energy possible for its composition, its formation will give out heat.

So while DREADCO's chemists wrestle with thermodynamic equilibria in rubbish, its chemical engineers are making Rumus in practice. They are compacting carefully defined mixtures of plastic bags and bottles, old newspapers, tin cans, broken china, disposable nappies and takeaway-meal residues at high temperatures and pressures, measuring the heat evolved and studying the product. From the results, they hope to design a pilot plant to carry out this self-heating reaction automatically and continuously.

Raw Rumus will be an uninspiring, brownish solid. At first Daedalus hoped that it might be hard and strong enough for road-stone or breeze blocks. But he then recalled that a mixture always has a lower melting point than its ingredients. Rumus will be such a horrendous mixture that its melting point will be quite low. Inert, softish and yielding, it will be ideal for asphalt, roof sealants, caulking and the like. It may, of course, contain two or more intermixed phases of different melting point, thus being self-reinforced into a tough, useful solid.

But to command a wide and varied market, it will probably need a bit of modification. The incoming rubbish may have to be spiked with conditioners like sulphur, to vulcanize and toughen the organic components; or silicon, which should combine with metals, organics and inorganics, to merge otherwise immiscible phases. Once the process has been optimized, DREADCO's Rumus will solve the whole refuse disposal problem, and make a splendid profit into the bargain.

David Jones

On the edge of a wing

SIR—It is very striking how the wings of all species of the fruitfly *Drosophila* have a similar, elliptical shape, independent of their size. This suggests that there could be a standard growth mechanism with parameters that vary within narrow limits.

D'Arcy Thompson[1] showed that the shapes of organs of various plants and animals can be accurately described using geometric figures defined by simple equations. To characterize the variation in size and shape of *D. mediopunctata*'s wing, we tried to adjust an ellipse to its contour. When we overlayed the adjusted ellipse on a photograph of the wing, we were impressed by the nearly perfect fit (see figure).

Because the shape and size of an ellipse can be precisely described by two independent parameters (in our figure *SH* and *SI*), we can characterize the shape and size of a wing using the parameters of the adjusted ellipse. We did this when we analysed the wings of 17 females collected from the field and of 42 of their daughters raised in the laboratory, half at 16.5 °C and the other half at 20 °C. Comparing the three groups, we found no significant difference in the shape of their wings ($F=0.91$; $P>0.9$; d.f.$=2$, 56) although there were clear and significant differences in size ($F=18.81$; $P<0.001$; d.f.$=2$, 56); showing that, despite the great variation in size, there is little variation in the wing shape.

We also adjusted ellipses to the wings of a few animals of other species: *D. atrata*, *D. quadrum*, *D. hydei*, *D. mulleri*, *D. willistoni* and *D. melanogaster*. Similarly, we examined published photographs of the wings of 17 *Zygothrica*[2] species and of 16 species and 40 hybrids of the picture-winged Hawaiian *Drosophila*[3]. In all cases, there was a good fit for the adjusted ellipses.

Together with use of genetic information available for *Drosophila*, the use of ellipses opens the possibility of new experimental investigations on questions related to size and shape. These could include evolution in species of *Drosophila* and related genera, environmental and genetic factors and the effects of selection for size on shape. We suspect that the wings of other insects may also be described by simple geometric figures, although not necessarily ellipses.

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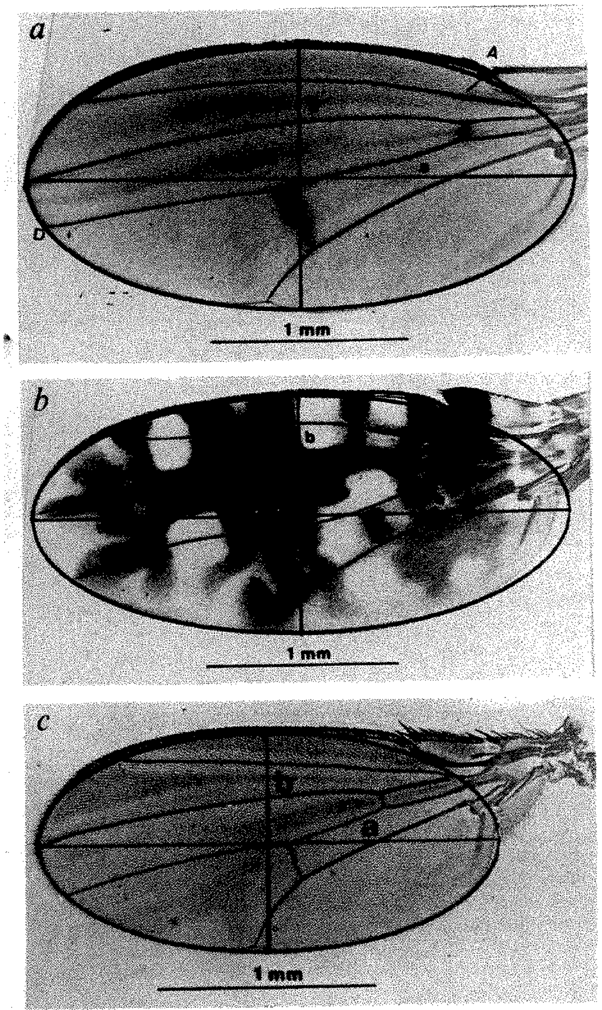


a, Wing and adjusted ellipse of *Drosophila mediopunctata* ($a=1.398$ mm, $b=0.653$ mm, $SI=\sqrt{ab}=0.955$ mm, $SH=0.4668$, $r=0.9998$). *b*, Wing of *D. quadrum* ($a=1.4156$ mm, $b=0.612$ mm, $SI=0.931$ mm, $SH=0.4324$, $r=0.99933$). *c*, Wing of *D. melanogaster* ($a=1.054$ mm, $b=0.496$ mm, $SI=0.723$ mm, $SH=0.4707$, $r=0.9996$). We took at least 20 points along the outline of the wing (using the internal edge of the marginal vein from A to D). We fit the general equation of the ellipse to the cartesian coordinates of these points using a least-squares procedure. Solving this equation for the *x*-observed values, we calculated a correlation (r) between the observed and expected *y*-values, and the centre and orientation of the ellipse found. Through a translation and rotation of the coordinates system, the general equation was transformed to $x^2/a^2+y^2/b^2=1$, where a and b are the radii of the two director circles of the ellipse. Their geometric mean is the size parameter ($SI=\sqrt{ab}$); the shape parameter is the proportion of the smaller to the larger radius ($SH=b/a$).

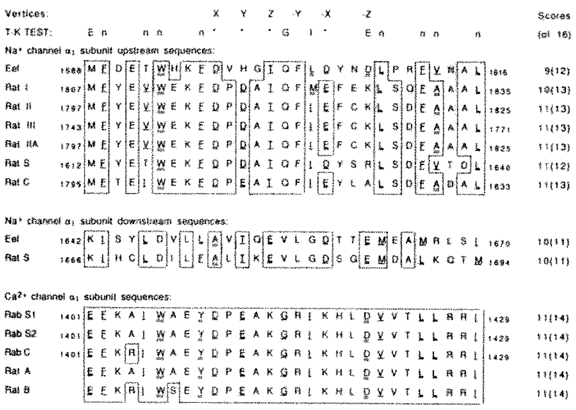
Channel hands

SIR—Calcium-binding proteins are grouped into several classes, one of which consists of intracellular transducers of calcium signals. These calcium-modulated proteins contain sequences of 29 amino acids, the EF hand which forms an α-helix, a calcium-binding loop and a second α-helix[1].

The deduced amino-acid sequences of many voltage-gated ion-channel proteins have been reported recently. Sodium-channel α_1 and dihydropyridine-sensitive calcium channel α_1 polypeptides have recognizably similar amino-acid sequences, both of which probably evolved by two gene duplications from a polypeptide resembling the *Drosophila Shaker* gene product, a voltage-gated potassium channel[2,3]. In the course of work on brain calcium-binding proteins, I found that all three calcium-channel α_1-subunit and seven of eight published sodium-channel α_1-subunit sequences contain one or more putative EF hands. The exception is the *Drosophila* DSC locus[4], but another probable *Drosophila* sodium-channel α_1-subunit which has been partially sequenced may have the conserved EF hand[5]. By contrast, none of the 10 potassium-channel sequences so far published contains apparent calcium-binding sites.

All the sodium-channel α_1-subunit sequences except the *Electrophorus* sequence contain a conserved region which scores 10 or 11 in the Tufty–Kretsinger test and all scores can be improved to 12 or 13 by allowing plausible test modifications described for other EF hands. Other criteria for calcium binding[5], such as the presence of three acidic ligands and the absence of more than one serine or threonine serving as a ligand in the calcium-binding loop, are fulfilled by these sequences. Critical positions are better conserved than are noncritical ones. Polypeptides from *Electrophorus* electric organ and rat skeletal muscle also contain a putative EF hand downstream.

The calcium channel α_1-subunit sequences score higher in the EF-hand test than do the sodium-channel sequences and they have more characteristics of EF hands, particularly the specified I, G and first E. The sequences of the rat aorta and brain calcium-channel α_1



Na⁺ channel α₁ subunit upstream sequences:

		Sequence		Score
Eel	1588	MEDETWHKEDVHGIQFLDYNDLPREVNAL	1616	9(12)
Rat I	1807	MEYEVWEKEDPDAIQFMEFEKLSQEAAAL	1835	10(13)
Rat II	1797	MEYEVWEKEDPDAIQFIEFCKLSDEAAAL	1825	11(13)
Rat III	1743	MEYEVWEKEDPDAIQFIEFCKLSDEAAAL	1771	11(13)
Rat IIA	1797	MEYEVWEKEDPDAIQFIEFCKLSDEAAAL	1825	11(13)
Rat S	1612	MEYETWEKEDPDAIQFIDYSRLSDEVTDL	1640	11(12)
Rat C	1795	METEIWEKEDPEAIQFIEYLALSDEADAL	1833	11(13)

Na⁺ channel α₁ subunit downstream sequences:

		Sequence		Score
Eel	1642	KISYLDVLLAVIQEVLGDTTEMEAMRESI	1670	10(11)
Rat S	1666	KIHCLDILEALIKEVLGDSGEMDALKQTM	1694	10(11)

Ca²⁺ channel α₁ subunit sequences:

		Sequence		Score
Rab S1	1401	EEKAIWAEYDPEAKGRIKHLDVVTLLRRI	1429	11(14)
Rab S2	1401	EEKAIWAEYDPEAKGRIKHLDVVTLLRRI	1429	11(14)
Rab C	1401	EEKRIWAEYDPEAKGRIKHLDVVTLLRRI	1429	11(14)
Rat A		EEKAIWAEYDPEAKGRIKHLDVVTLLRRI		11(14)
Rat B		EEKRIWSEYDPEAKGRIKHLDVVTLLRRI		11(14)

Amino-acid sequences in sodium- and calcium-channel α_1 subunits examined by the Tufty–Kretsinger EF-hand test. Ligand vertices for calcium coordination (X, Y, Z, -Y, -X, -Z) as described in ref. 1 are indicated. In the test sequence, E, G and I are specified where indicated: *n*, a hydrophobic residue (L, I, V, F, M); asterisk, an oxygen-containing amino acid (D, N, E, Q, S, T). Amino acids satisfying the test are underlined. Plausible modifications to the test are double-underlined with the increased scores in parentheses. Origins of these sequences are as follows: Eel, *Electrophorus electricus* electric organ[9]; Rat I and rat II, rat brain[10]; Rat III, rat brain[11]; Rat IIA, rat brain[12]; Rat S, rat skeletal muscle[13]; Rat C, rat cardiac muscle[14]; Rab S1, rabbit skeletal muscle[15]; Rab S2, rabbit skeletal muscle[16]; Rab C, rabbit cardiac muscle[17,18]; Rat A, rat smooth muscle (W. J. Koch, unpublished); Rat B, rat brain (A. Hui, unpublished).

subunits in this region are very similar to the rabbit skeletal muscle sequences (W. J. Koch and A. Hui, unpublished data). The putative EF hand linked to the IVS6 region 25 amino acids upstream is one of the most highly conserved areas in the entire polypeptide with only two noncritical (for EF hand formation) changes. No downstream EF hands were detected in these sequences. Overall, only 2 of 29 amino acids are identical across all 12 sequences, so it is surprising that the structural features (supported by secondary-structure predictions; ref. 6 and unpublished data) of a particular kind of calcium-binding site should be retained.

The high degree of conservation in these sequences suggests that the putative EF hand(s) are important in channel structure and/or function. This region is internal and probably close to the lip of the channel. Because these sequences occur in sodium and calcium channels and not potassium channels, calcium binding could be involved in some aspect of depolarization and not repolarization or facilitation of ion permeation. Perhaps calcium binding is part of an inhibitory mechanism to close or inactivate these channels consequently limiting depolarization and cation influx. If, on the other hand, the sodium-channel sequences do not bind calcium ions with micromolar affinity, then the calcium-channel sequences could be the high-affinity internal calcium binding site which facilitates ion permeation[7,8].

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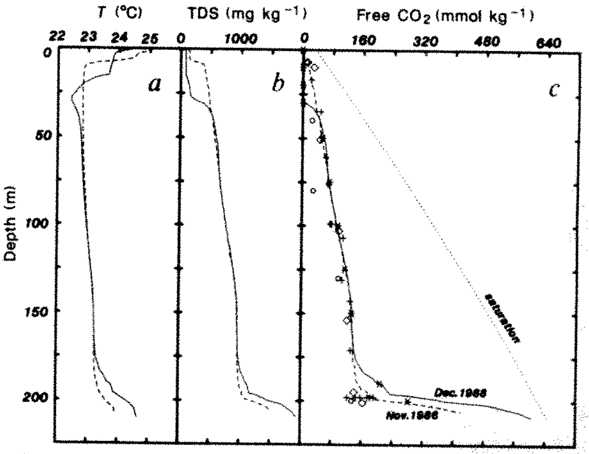
Gas discharge at Lake Nyos

SIR—An outburst of lethal gas from Lake Nyos killed about 1,700 people on 21 August 1986[1]. It has been confirmed[1,2] that the gas was almost pure CO_2 of magmatic origin, but the volume of gas released, the mechanism of the sudden release and the likelihood of a future recurrence remain open questions. We conducted a survey in December 1988 to measure temperature, conductivity and CO_2 profiles in the lake, which has enabled us to estimate the influxes of heat and dissolved materials, and to estimate a recurrence time for such a gas discharge.

We measured temperature and conductivity profiles with a CTD (conductivity–temperature–depth) profiles; gas concentrations were measured by collecting water samples from various depths using a 1.4-l Niskin bottle with a plastic gas bag attached[2]. Samples below 50 m contain constant relative concentrations of Mg^{2+}, Fe^{2+}, Ca^{2+}, Na^+, NH_4^+, K^+, HCO_3^- and Si. These components were summed for each sample to convert measured conductivity to concentration of total dissolved solids (TDS). Temperature and TDS profiles in December 1988 are compared with those obtained in November 1986[3] (*a* and *b* respectively in figure). The increase in both temperature and TDS below 165 m is significant, and supports the view that the lake is fed by an inflow of warm, CO_2-charged, mineralized water[2,4,5]. Integrating the temperature and TDS profiles below 165 m gives heat and TDS fluxes of 0.36 MW and 1,064 tonne yr^{-1}, respectively.

Complete sampling of both gas and water is difficult because the deep water contains a very high concentration of free CO_2 even after the 1986 gas release. We have developed a simple syringe sampler with which CO_2 can be fixed *in situ* in a solution of NaOH. Sampling for five depths between 50 m and 150 m gave total CO_2 concentrations that increase linearly with TDS. Free CO_2 concentrations, given by the difference between total CO_2 and HCO_3^- concentrations, are plotted in *c* of the figure together with data obtained between October 1986 and May 1987[2-4]. Our free CO_2 data for the mid-depth water (50–165 m) were smoothed using the linear CO_2–TDS relationship. Over the two years, there has been no change in free CO_2 concentration at mid-depths.

For depths greater than 175 m, the high CO_2 concentrations made proper sampling difficult. Fortunately, however, we were able to recover 7.08 l of gas from a depth of 190 m comprising over 99.8% CO_2 (refs 5,6) which completely exsolved into the collection bag, indicating a free CO_2 concentration of 209 mM. In the sample from 200 m, the exsolved gas exceeded the capacity of the 10-l bag, implying that the free CO_2 concentration was higher than 283 mM. The free CO_2 concentrations at 190 and 200 m indicate that the CO_2 /TDS ratio is higher below



a, Temperature profiles in Lake Nyos. Sold line: our data for 16 December 1988. Dashed line: data for 8 November 1986[3]. *b*, TDS profiles in Lake Nyos calculated from the correlation between CTD conductivity (temperature-corrected) and TDS in the sampled water. *c*, Measured and estimated free CO_2 concentration in Lake Nyos. Measured concentrations indicated by circles (October 1988[2]), squares (November 1986[3]), crosses (January, March and May 1987[4]) and stars (December 1988, our data). The 200-m data point for December 1988 indicates an approximate minimum free CO_2 concentration (see text). Solid (our data) and dashed (ref. 3) lines smoothed free CO_2 profiles based on the TDS profile and CO_2–TDS relationship. Dotted line, saturated concentration[8] of free CO_2 for the pressure and temperature conditions of the lake.

165 m than at mid-depths. We assume that much of the total dissolved CO_2 was released in the 1986 gas outburst and that non-gaseous components were retained; thus the deep-water CO_2/TDS ratio is higher because it is being replenished from a bottom spring rich in CO_2.

If CO_2 concentrations in the deep water result from mixing of mid-depth water and bottom spring water, the CO_2–TDS relationship for the deep water can be assumed linear, with a slope that can be estimated from data points at 150 m and 190 m. We used this slope to convert the TDS profile to the CO_2 concentration profile in the deep water (*c* in figure). We analysed Tietze's CTD data[3] in a similar fashion. The smoothed free CO_2 profile (see *c* in figure) suggests that the bottom water is approaching CO_2 saturation. We calculate an average free CO_2 flux over the past 25 months of 515×10^6 mol yr^{-1} (0.012 km^3 STP CO_2 yr^{-1}). Based on this CO_2 flux and a revised bathymetry[7], the hypolimnion (depths below 30 m) of Lake Nyos may become saturated with free CO_2 within about 50 years if the estimated CO_2 flux remains unchanged. Frequent measurements of water and gas compositions of the lake would contribute to more accurate evaluation of CO_2 fluxes, essential to assess the possibility of a future gas release.

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Natural habitat of coelacanths

SIR—Fifty years after the discovery of living coelacanths, these ancient fish are threatened with extinction within two decades[1]. Can this famous fish be protected and saved?

Almost all (98.6 per cent) of coelacanths have been caught along the west coast of Grande Comore, Western Indian Ocean (the site where most of these fish have been captured)[2]. Hardly any have been found on the east coast. Last year, we carried out a submersible operation to study the extent of the population and surveyed 9 per cent of the area thought to be the coelacanths' habitat (see map).

Coelacanths, shy but present.

During daytime, coelacanths are cave dwellers and aggregate in small peaceful groups, the number of members changing daily. Each coelacanth occupies a home range of at least 8 km of coastline. At this site, there are several occupied caves between 180 and 210 m in depth. At night, coelacanths leave their caves and hunt singly for fish. The number of caves seems to limit their distribution. Below 220 m depth, caves are rare and prey fish are less abundant. On the other hand, temperature dependent oxygen saturation of the blood[3] probably prevents the occupation of shallower warmer waters.

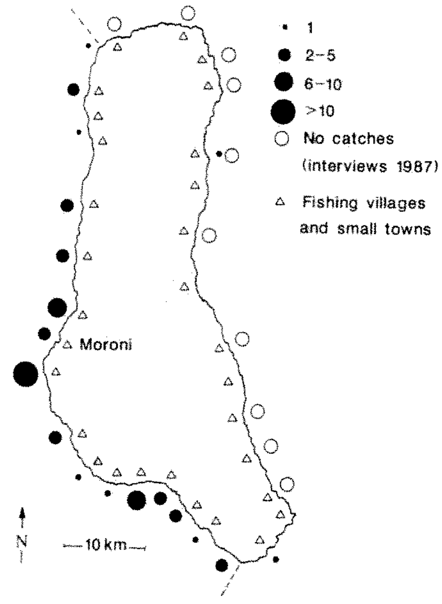
We found 38 individuals. We recorded 19 as residents since they were repeatedly encountered. Assuming that this sample is representative, the total population of the west coast can be estimated as 210 individuals. By an alternative method, repeated observation of 6 occupied caves revealed an average of 13.5 coelacanths or 150 individuals, for the entire coast. These are first estimates for the coelacanth population at Grande Comore. The small differences between both estimates suggests that these are reliable numbers. If the highest number of observed coelacanths per cave is taken as an indication for cave saturation, 370–520 individuals would be the habitat's carrying capacity at Grande Comore, which is indeed a very small number.

Among the encountered individuals, only two were 60–80 cm long, and 5 per cent are smaller than 100 cm. Size at birth is probably 40 cm. The two juveniles were observed together with one large female, suggesting that their habitat is similar to that of adults. Official catch records reveal that 3 of 43 individuals (7 per cent) were smaller than 100 cm. Low numbers of juveniles can result from low birth rate and/or high juvenile mortality. Coelacanths are ovoviviparous, fecundity probably being low and the gestation period more than 12 months. Nothing is known about longevity, growth or age at maturity, or size, age structure and sex ratios of populations. Although our present knowledge of life-history parameters is incomplete, coelacanths seem to be highly K-selected organisms with a low production of offspring and an apparently high longevity. Therefore, the small population size we observed should be a cause for concern.

Official catch records for the past 28 years from Grande Comore show that 2.6 ± 1.5 (s.d.) individuals (or 1–1.5 per cent of the total population) are annually eliminated by human fishing. Over the years, the catch rate of coelacanths has remained stable although local fisheries have expanded. This could indicate that the coelacanth population is already in decline. But increased fishing efforts do not necessarily imply that there has been proportional increase in fishing for coelacanths. Coelacanths are caught at night from small outrigger canoes as an incidental bycatch, and we have found no evidence that night fishing has increased. Furthermore, the rapid developments of the pelagic fishery outside the coelacanths' habitat do not harm the coelacanth population. If the estimations of the carrying capacity are correct, then the low population size could have existed for centuries. For many years coelacanths have been caught in very small numbers, and therefore the shift from traditional to modern fishery has not affected the population.

In the future, we should carefully monitor the number of coelacanths in this region. Details of life-history parameters need to be studied to predict changes in the population. Coelacanths are rare, and concentrated international efforts should be made to protect them. Threats to their survival might arise from commercial interests such as public aquariums.

Since September 1989, trade of living coelacanths is strictly forbidden by a decree


• 1
● 2–5
● 6–10
● >10
○ No catches (interviews 1987)
△ Fishing villages and small towns
Moroni
N
—10 km—

Map of Grande Comore (capital Moroni) with locations of coelacanth catches (filled circles) and distribution of coastal fishing villages and small towns (open triangles). The west coast between Mitsamiouli and Chamboini (dotted lines) is the coelacanth habitat.

of the late president of the Comores. In October 1989, coelacanths were upgraded to schedule 1 of the conservation charter CITES. This might ensure that future generations can rejoice in these gentle and peaceful survivors of our own vertebrate history.

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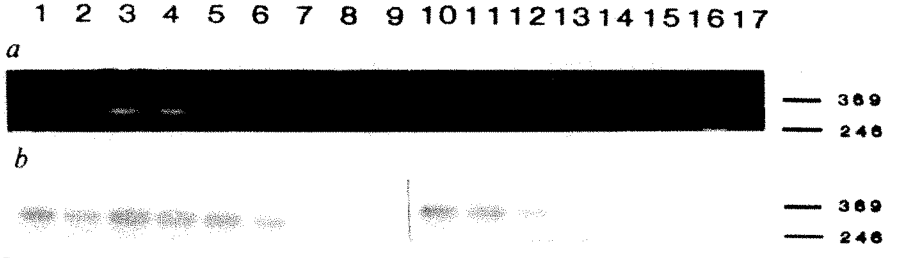
Improving PCR efficiency

SIR—The polymerase chain reaction (PCR) is a very sensitive *in vitro* DNA amplification method[1]. Therefore, carry-over of even minute quantities of reaction products can lead to serious contamination problems and false-positive results. Tidiness and adherence to a strict set of protocols can avoid disaster[2,3]. Methods which eliminate contamination by PCR products from previous amplifications are indispensable, especially for diagnostic applications. As recently reported in Scientific Correspondence[4], such contaminations can be eliminated from reaction mixtures by ultraviolet treatment before addition of target DNA.

We have developed an alternative method in which treatment of individual reaction mixtures before adding template DNA and *Taq* DNA polymerase with DNaseI or restriction endonucleases that cut internal to the pair of amplification primers prevent amplification of contaminating DNA. As a model system, we used the amplification of a 275-base pair fragment of the *elt* gene of enterotoxigenic *Escherichia coli* ATCC 37218 (ref. 5). We added 5 – 50,000 lysed bacterial cells to individual reaction mixtures (excluding *Taq* DNA polymerase), followed by 0.5 units of DNaseI or 10 units of *MspI*. After incubation and subsequent inactivation of enzymes, we added *Taq* DNA polymerase and performed 35 cycles of PCR. As shown in the figure, *MspI* treatment reduces contamination by a factor of 5 to 10 without impairing the efficiency of the PCR. DNaseI treatment reduces contamination by a factor of 1,000. Comparison of lanes 1 and 10 shows that the efficiency of PCR is not reduced by this treatment as primers are not significantly attacked by DNaseI under the conditions chosen. The decontamination conditions can easily be optimized together with optimization of PCR components.



Decontamination efficiency of DNaseI or *MspI* treatment in PCR. (See ref. 5 for standard methods.) Reaction mixtures (excluding *Taq* DNA polymerase) which contained lysed bacteria as artificial contaminations were treated with 0.5 U DNase I for 30 min at room temperature or with 10 U *MspI* for 1 h. After incubation, enzymes were inactivated by boiling, *Taq* DNA polymerase added and 35 cycles of PCR performed. *a*, ethidium bromide-stained 1.5% agarose gel; *b*, Southern blot of 10 μl PCR product. Lanes: 1, 50 *E. coli* ATCC 37218, no DNaseI or *MspI* treatment of reaction mixture; 2, reaction mixture *MspI* treated before adding 50 bacteria; 3–9, contamination of reaction mixture with 50,000, 5,000, 500, 50, 10, 5 and 0 bacteria, respectively, *MspI* treatment; 10, reaction mixture DNase I treated before adding 50 bacteria; 11–17 contamination of mixture with 50,000, 5,000, 500, 50, 10, 5 and 0 bacteria, respectively, DNase I treatment.

The efficiency of restriction-enzyme digestion can be increased by adding more than one enzyme and by increasing the enzyme concentration as well as the incubation time. The sequence specifity of the restriction-enzyme treatment can be circumvented by using a cocktail of enzymes that recognize tetranucleotide target sequences. The main advantage of this method is its general applicability based on the use of DNaseI, a cheap enzyme requiring no sequence information between amplification primers.

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Too soon for consensus?

SIR—Crumpton and Dedman in their Scientific Correspondence (*Nature* **345**, 212; 1990) proposed a common nomenclature system for a group of proteins presently referred to as lipocortins, annexins, calpactins and so on. Several of us, including those who first identified, isolated and cloned several members of this family, do not agree fully with these changes.

We have no objection to the use of the general designation of annexin to describe the group. But because there is no clear, universally accepted function for any of the proteins we believe that a new nomenclature is premature. Because each family member probably has a specifically tailored function, we suggest, at least in the interim, that a common universally accepted name for each protein (calpactin, endonexin and so on) would simplify matters.

One member of this family has been termed lipocortin-1 in most papers published over the past 2 years. The name lipocortin is already a result of an attempt to simplify the nomenclature and is reasonably descriptive of the protein's properties. In view of this fact, we feel that it would be an additional burden to impose yet another name — annexin-1.

We would like to make a plea for an open system of nomenclature until more research has shown us the most probable biological roles of this perplexing yet fascinating family of proteins.

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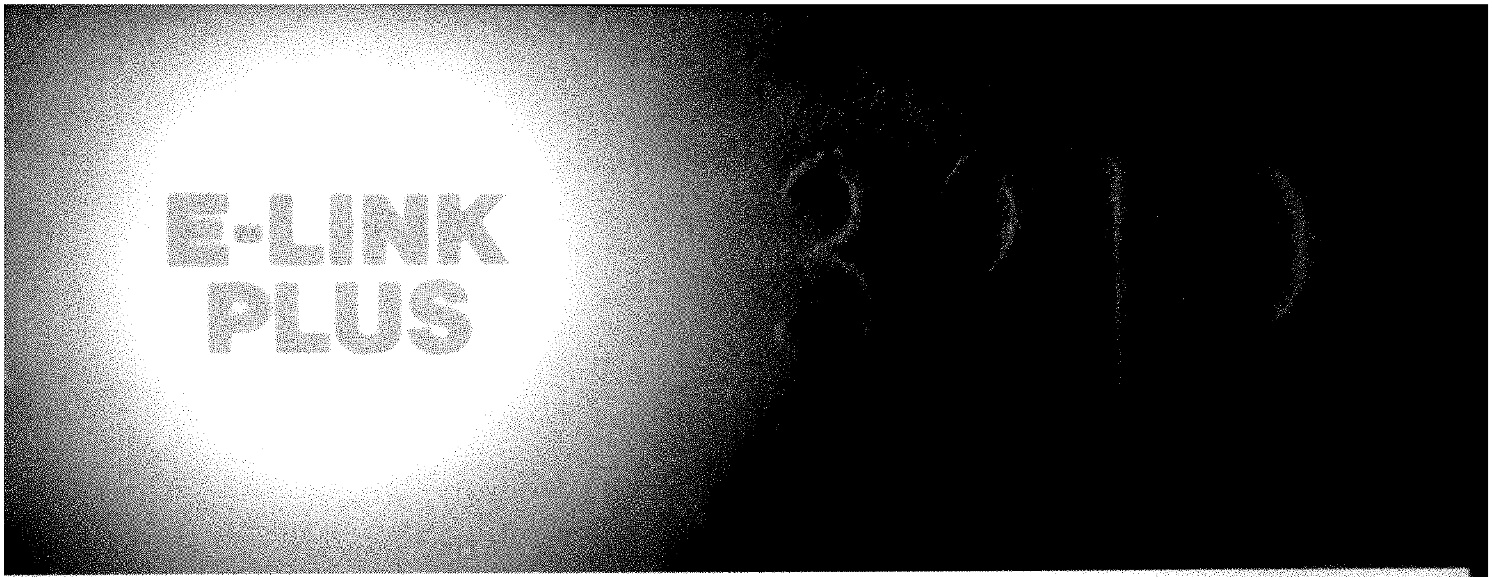

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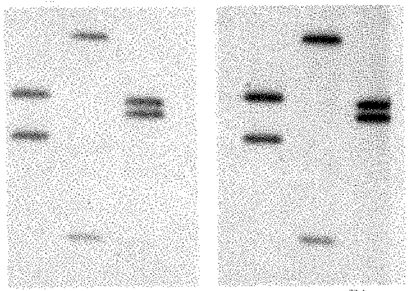
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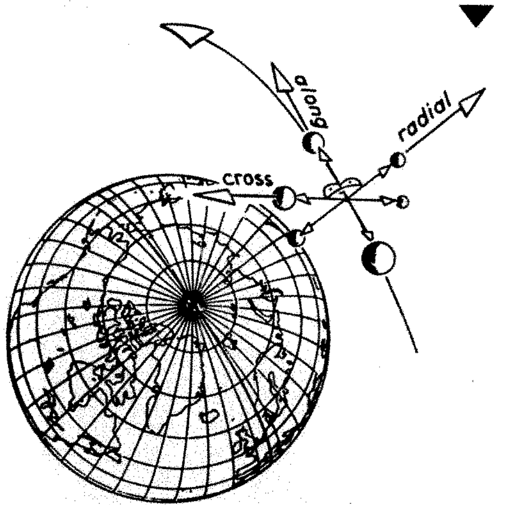

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A definite maybe

E. I. Hamilton

Deadly Deceit — Low Level Radiation, High Level Cover-Up. By J M Gould and B A Goldman. *Four Walls Eight Windows, New York: 1990. Pp. 213. $19.95.*
How Safe is Safe? Radiation in our Daily Lives. By Barrie Lambert. *Unwin: 1990. Pp.275. Pbk £7.99.*

Two popular concepts in radiological protection are that all ionizing radiation is harmful to living cells and that there is a linear relationship between dose and effects. But some recent studies indicate that these concepts may not apply to people exposed to low doses of radiation. The significance of radiation risks is largely a polemical issue — for low doses (1–10 millisieverts per year) the evidence for presumed effects is largely of a statistical nature and is not always supported by epidemiology.

Gould and Goldman present a statistical evaluation for excess deaths in the United States which they relate to exposure from low-level radiation (the levels are not given) following releases of radioactivity into the environment from global fallout, nuclear accidents and from nuclear establishments. They consider reported excess deaths in the United States to be high — for example, 40,000 after the arrival of Chernobyl fallout. Those most susceptible are the very young, the very old and those suffering from infectious diseases. A comprehensive demographic breakdown of mortality and morbidity statistics is not provided by Gould and Goldman, neither do they mention the radiation dose received by individuals, nor the radiobiological effectiveness of the radiation. Instead they simply relate excess deaths with concentrations of radionuclides in air, water and food. The apparent delay between insult and effect (death) is usually only a few months; such a feature is unknown in radiological protection except following exposure to extremely high doses of radiation. The cause underlying the effects of exposure is considered to be an impairment to the immune system caused by free radicals formed in tissues following the passage of radiation. The authors comment on the presumed high levels of fallout in central Africa in the 1950s and the emergence of AIDS (or should this be HIV?), together with the recent increase in other immune-response diseases such as *Candida albicans* infections, Lyme disease, chronic Epstein–Barr virus infections, septicaemia and an impairment of mental and scholastic achievement, all of which the authors say have increased in the United States since nuclear-weapons testing first started.

The authors use naive statistics and an inadequate database, paying insufficient attention to the role of demographic variables common to the subset of exposed individuals in relation to the general population. They do not consider modern statistical approaches to complex issues such as hazard models developed by Cox. They do not discuss possible effects of low-level doses from natural sources, or localized enhanced levels such as those associated with the mining of uranium ores, for example at Katanga, central Africa or within the Wismuth–Jochamstal region of central Europe. Neither have they considered the morbidity and mortality data following exposure to radon in houses. All these sources are relevant to any consideration of low-dose effects from ionizing radiation. The authors have extrapolated results of recent animal experiments far beyond that which is reasonable, even though some studies do indicate an involvement between radiation and the immune system. They also cite examples where federal agencies are accused of suppressing or manipulating data, a practice which is not unique to radiation issues.

Considering all the industrial products now present in the environment, some of which are known to constitute a risk to health (somatic and genetic), singling out low levels of radiation as the most important cause of excess death and disease is not justified. Perhaps today, through poorly understood interactions and the prevasive distribution of chemicals (both man-made and natural) in the environment has produced a matrix in which transferable mutagenic changes can occur, and this could be one of the costs of technological advance for which technical remedies will eventually need to be forthcoming.

Geoff Franklin/Network

Food for thought — picnicking at Sellafield nuclear reprocessing plant.

The future of the nuclear industry, and especially the production of electricity, requires a deeper understanding both by the general public and governments. The many controversial issues need to be discussed openly and both the risk and advantages set in perspective. Lambert provides an excellent account which is intended to allay fears and to explain some of the controversial issues, based upon scientific evidence rather than scientifically baseless claims. All the main issues, ranging from measurement of radiation, its effects, estimation of risks, nuclear reprocessing and discharges of radionuclides into the environment, are discussed. The pros and cons of the various arguments are stated in a fairly impartial way; those areas where uncertainties still exist and when policies have been suspect are highlighted.

Radiobiological aspects are treated in a comprehensive manner but the discussion of environmental distributions of radionuclides is weak, which is a pity as many pressure groups focus their concern upon the presence of man-produced radionuclides in the environment. For the general public it is important to get the message across that today there is a sound basis for proliferation of nuclear power; there are no insurmountable scientific problems in the disposal of waste products to the sea or land. There are risks associated with all technologies, but there are also considerable advantages — hence a balance has to be achieved very soon.

Lambert provides the scientific framework upon which evaluations and judgements can be made by those with limited knowledge of nuclear matters, by contrast to Gould and Goldman who present areas of concern which are not based upon proven scientific evidence, but rather the extrapolation of controversial findings well beyond any reasonable level of acceptance. Nevertheless, there are some facts in the fiction — but of course this is common to many areas of controversy, especially when evaluating man's impact on the environment in relation to technological and social evolution.

In the short to medium term, there are no viable alternatives to the generation of electricity by nuclear means, neither are there any serious technological difficulties involved in the process. To expect a no-effect relation between technology, man and the environment is not reasonable. Overall, the benefits of accepted technology outweigh the disadvantages. It is to be hoped that the nuclear debate will become more open and informed so that the advantages and disadvantages can be properly evaluated. ☐

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Cold fusion and other matters

FOR many years, trenchant observations on the affairs of science in the United States have been provided by the legendary Grant Swinger, director of the mythical Center for the Absorption of Federal Funds. Here Dr Swinger converses with Daniel S. Greenberg of *Science & Government Report*, on 1 June 1989.

Q. How do you see the Bush Administration shaping up in the science-policy area?

A. That's best judged after they take office.

Q. They've been in for over four months.

A. Four months? As long as that. It is difficult to keep track of time in the absence of activity. But I can say that the general atmosphere in the country and in Washington is favourable to innovative approaches.

Q. Example.

A. Cold fusion. An exemplary case in the best tradition of the Center for the Absorption of Federal Funds. If I have any concerns, they're directed at my own institution for not doing it first. Think of it: no publication, just a press conference. And they get $5 million from the Utah legislature. Jim Fletcher, the ex-chief of NASA, joins up with them, and over a hundred corporations line up and plead for a piece of the action. It's on the covers of *Time* and *Newsweek*, and Congress invites them in to talk about $25 million. And if there's anything there, no one can find it! This is a triumph. We've worked for years to get the Congress, the press, and the public to this stage.

Q. Very unusual events.

A. Actually, we've pulled this off in medical research many, many times. There's almost nothing that hasn't been reported cured or on the brink of a cure. But this has hardly ever been done in the physical sciences. The closest thing now going is the search for extra-terrestrial life, but that's never got beyond a small scale.

Q. On cold fusion, there was some scepticism at the outset.

A. That's bound to happen when you try to undercut other researchers in a field. The main opposition came from the hot-fusion tokamak crowd — very innovative people when it comes to protecting their territory. After the first report about cold fusion came out, they advised Congress not to put any money into it until it's proven.

Q. That seems to be a prudent approach.

A. Prudent to wait until it's proven? Hot fusion has been running for 30 years without anything proven. And they're now at $400 million a year.

Q. They feared competition?

A. It was potentially very embarrassing, very threatening, this tabletop stuff for a mere $100,000. But do you know the worst of it?

Q. What?

A. The worst of it was that these fusion researchers shouted that they used their own money. As far as I'm concerned, that's unforgivable. They're cannibals. Whether or not cold fusion works is a minor matter. But using their own money. The precedent, I mean —

Q. Please, calm down, Dr Swinger. What are you planning at your centre?

A. We have a number of activities. Inspired by recent events, we're working on an alternative to the superconducting super collider.

Q. What will that be?

A. Table-top particle acceleration. Our motto is: "Fifty-three inches is better than 53 miles. One in each state." The politics are sound, even better than our old proposal for a transcontinental linear accelerator that would run across a dozen or so states That would have brought in 24 senator and maybe a hundred congressmen.

Q. Is table-top particle accleratio feasible?

A. We're going about this in the righ way to find out. First, we're setting up task force to plan a workshop preliminar to holding a conference. Next comes a cal for papers. Then we'll circulate the proceedings for comment and issue a draf report. A small conference will follow t oversee preparation of the final report This is the standard way of approachin these things. We'd be criticized if we wen about it in any other fashion.

Q. If I may say so, table-top particl acceleration does seem too far-fetched t warrant all that effort.

A. This is an open-ended business. Opportunities can be anyplace. A lot o people thought Star Wars was too far-fetched to get a nickel. But there it is. And what about the manned space station?

Q. What about it?

A. It's terrific. No one knows how much it will cost or what it's supposed to do. But it's going ahead. That's an ideal project, in our view.

Q. What do you foresee as new growth areas?

A. There's a lot of talent out there working up new possibilities. We were worried about the Department of Energy losing that old entrepreneurial spirit, but they reassured us all by cooking up the mapping of the human genome. It's not really their business, but that lit a fire under NIH and they've moved into the genome trade. Of course, it also took a little nudging about the Japanese rushing into this before Congress came across with the money. But bless the Japanese. They've arrived just as the steam was going out of the warnings about how we have to beat the Russians in this or that. What would we do without the Japanese? Without them, superconductivity research would be in the poor house.

Q. What else do you see ahead for growth in science and technology?

A. The greenhouse effect is bound to be a gold mine. And we see a lot of forthcoming activity devoted to agonizing over scientific priorities: conferences, white papers, congressional hearings, and all the usual stuff. It's a small industry all by itself.

Q. In this period of economic challenge, well-chosen scientific priorities are very important for making the best use of valuable scarce resources, aren't they?

A. What?

Q. I was noting the importance of well-chosen scientific priorities.

A. I see. Excuse me, I'm late for a meeting. ☐

The Grant Swinger Papers, 2nd edn. *Science & Government Report, 6226 Northwest Station, Washington, DC 20015: 1990. Pp. 40. $8.95 ($10.95 overseas orders).*

Shifting mosaic

Peter D. Moore

Dynamic Biogeography. By R. Hengeveld. *Cambridge University Press: 1990. Pp. 249. £30, $54.50.*

"DYNAMIC" is an interesting word to use in the title of a book. It suggests that the subject may well be perceived by the potential reader as static, if not stagnant, and that the author intends to dispel such an illusion. This is partially true of biogeography. There was certainly a time, perhaps just two decades ago, when biogeography was an essentially descriptive subject, being concerned mainly with the classification of distribution patterns of species and communities, but with little emphasis on the processes involved in the establishment of pattern. But this has changed in more recent times, and one might reasonably expect any modern text on biogeography to be "dynamic".

It is the insertion of the time element that converts a static approach into a dynamic one, and this must be the first test of the claim inherent in the title. Hengeveld certainly passes this test as he sets out to examine both temporal and spatial variation in biogeographical patterns and to explain underlying causes, emphasizing climatic and human induced factors. One of the main problems of biogeography, however, is what Hengeveld terms the "idiosyncratic properties of species distributions". It is difficult to make general statements on the basis of the wide range of patterns and the spread of causative factors found among plant and animal species. The detection of pattern within the patterns clearly demands sophisticated analytical techniques, and the first part of the book is concerned with the application of multivariate statistics to the classification and ordination of species-distribution data. This initial process in biogeographical methodology provides, in Hengeveld's view, an efficient system for hypothesis generation, such hypotheses usually relating either to the past history of the taxa, or to their ecological demands and limitations. But the two types cannot be clearly separated, for the physical and biological environment is itself not static, so ecological demands simply place limits on species distribution within the ever-rolling historical framework.

The logical progression of this line of thought would take us into a consideration of changes in the ranges of selected taxa over various timescales, but the author chooses to leave this development until later in the book and to insert a section on geographical trends in species richness and other biotic features. If one reads the book sequentially, then this tends to disrupt a well-constructed argument — but the topic is one of considerable biological interest and certainly demands coverage. There are some biogeographical generalizations that are supra-taxonomic, such as climatic relationships to diversity, vegetation architecture, life form, leaf form and so on, that can assist in an understanding of the spatial arrangements of the world's biota. Hengeveld tackles this section of the book by choosing specific examples to illustrate the general development of biogeographical thought in these fields, such as gradients of bird richness in North America, species "nesting" and its relationship to refugia hypotheses, C3/C4 photosynthetic strategies and climate, and many others.

The author finally returns to the theme of individual species' ranges and their analysis. He considers general features of population response curves to environmental variables and illustrates these with examples in which species boundaries can be related to climatic factors. On its own, however, such an approach might be regarded as static rather than dynamic, so Hengeveld concentrates on species for which there is adequate historical information from the fossil record so that the time factor can be incorporated into the model. The examples he uses, like *Tilia cordata*, are selected to illustrate the potential of this technique. But such relationships are rarely simple and neat, and in the final section of the book Hengeveld considers some of the complicating factors, such as the stochastic impact of weather and the lag that may exist between environmental change and species response.

The book provides a realistic account of what has been achieved by modern biogeography. But it also leaves me with the impression that the future of biogeographical development is largely in the hands of the ecophysiologist and the population geneticist, with some help from the statistician. I would say that the taxonomist and the palaeoecologist still have important parts to play in the assembling of data upon which testable hypotheses can be established.

This is a stimulating book, well written and arranged. There is a refreshing, logical line of argument that underlies the text and which leads the reader painlessly through the morass of information that typifies biogeography. It well justifies its claim to be dynamic. ☐

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Whatever you do, wherever you go, plants will have made some contribution — this is the message of *Plants for People* by Anna Lewington. The volume presents both well known and not so well known uses of plants; there are details of those that protect, cure, transport (reed boats above), entertain and the better known examples of those that clothe and feed us. The book, well researched and presented, illustrates almost all examples in full colour. Interesting information gleaned from the pages includes the story of the fine cloth made from pineapple leaves, the bamboo used for scaffolding and the anaesthetics from the Andes. Published by The Natural History Museum, London. Price is £19.95. ☐

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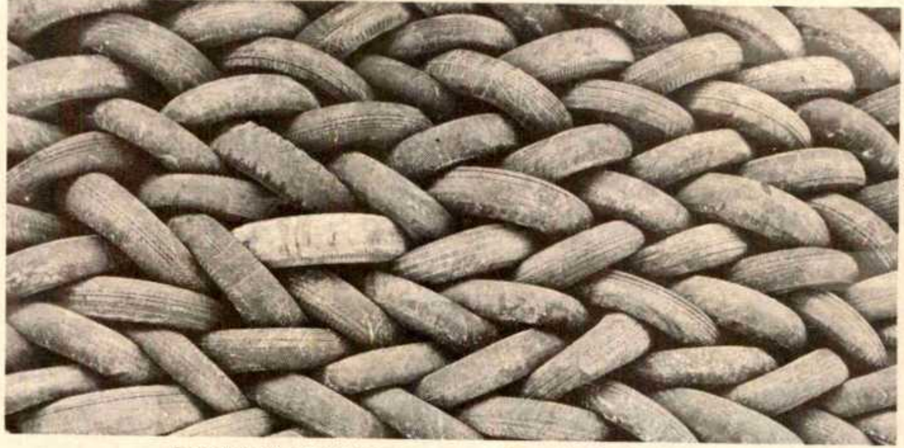
Trucking on

Paul Calvert

The American Synthetic Rubber Research Program. By Peter J. T. Morris. *University of Pennsylvania Press: 1989. Pp. 191. $34.95, £33.20.*

AT THE outset of the Second World War tyres were mainly made of natural rubber from Indonesia and Malaysia. To avoid a loss of supplies, both Germany and the United States put a huge effort into building a synthetic rubber industry. The best available rubber was a styrene–butadiene copolymer prepared by emulsion polymerization. This was first developed in Germany in 1929 and was adequate for car tyres but not good enough for truck tyres. There was no way to synthesize "natural rubber", *cis*-polyisoprene. From 1942 to 1956 the US Government spent $56 million on a well-organized research effort to ensure a domestic supply of rubber for car and truck tyres. The goals were then to set up efficient production plants, to improve the properties of the rubber produced and preferably to replace it with a better synthetic rubber.



Searching for new polymers — no alternative in the haystack

Morris has interviewed many of the participants in the scheme and asks whether the research and development programme was a success. His answer is a qualified "no". The overall production programme was a success in that $700 million was invested in 51 plants to produce 720,000 tons of rubber a year by 1945, but the research produced no major new rubber and when synthetic *cis*-polyisoprene finally came it was too expensive. The main participating companies were Goodyear, Goodrich, US Rubber and Firestone with Phillips Petroleum and General Tire on the sidelines.

It is fascinating to follow Morris to his conclusions. One reads a section thinking "Well, this aspect was not a success, but he has forgotten such-and-such a spin-off" and then sees that argument demolished in the next chapter. Morris divides research progress into incremental improvements, large improvements and breakthroughs. Incremental improvements in processes came continuously and were shared between the companies, but probably would have come anyway. Large improvements could mean a significant competitive advantage to a company so each one tried to keep these outside the programme and the pool of shared information. Breakthroughs can be very expensive and can upset a smoothly running bureaucracy and thus are to be avoided if possible. There really were none of these in the course of the programme.

The bulk of the $7 million university funding went to Illinois, Chicago, Minnesota and Cornell. Marvel's group at Illinois put a large effort into developing more than 100 new rubbers but none of them were better than butadiene–styrene. It is a characteristic of materials research that the first round of work often produces a material which is never bettered in subsequent projects. Flory at Cornell did a great deal of good polymer science but seems to have been regarded as extraneous to the main programme. The late 1940s and 1950s were a time of very rapid growth in polymer science and in the plastics industry. Morris argues that the academic contribution to progress by the rubber industry was small. There was mistrust of the universities by the companies. On the academic side, there was little knowledge of industrial problems and a lack of appreciation of short industrial timescales.

Many of the participants in the programme think of it as a great success. It is a bit unfair to criticize them for not finding an alternative rubber for truck tyres, for it is clear that there really wasn't one. The haystack had no needle in it.

Morris is not a polymer scientist, but there are few places where this really shows. An appendix summarizes polymer chemistry for the uninitiated. ☐

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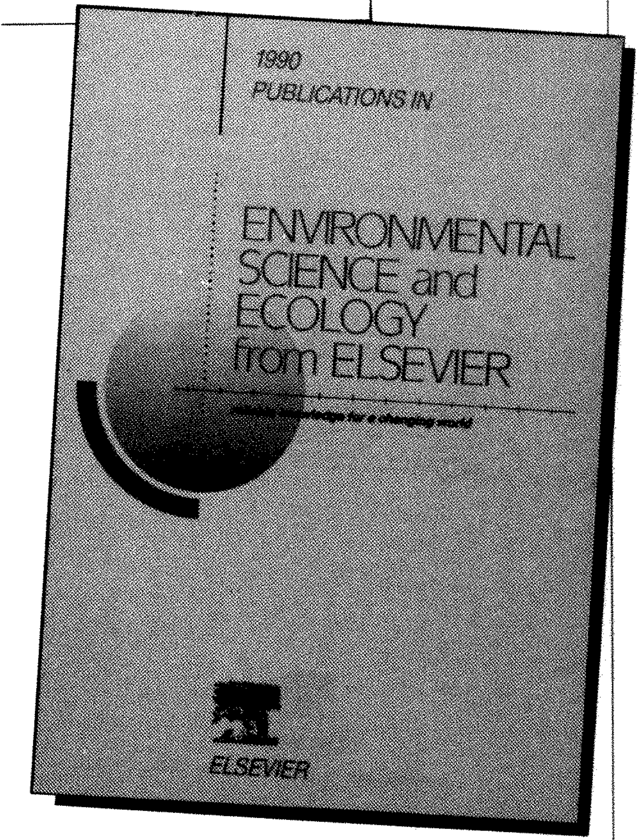
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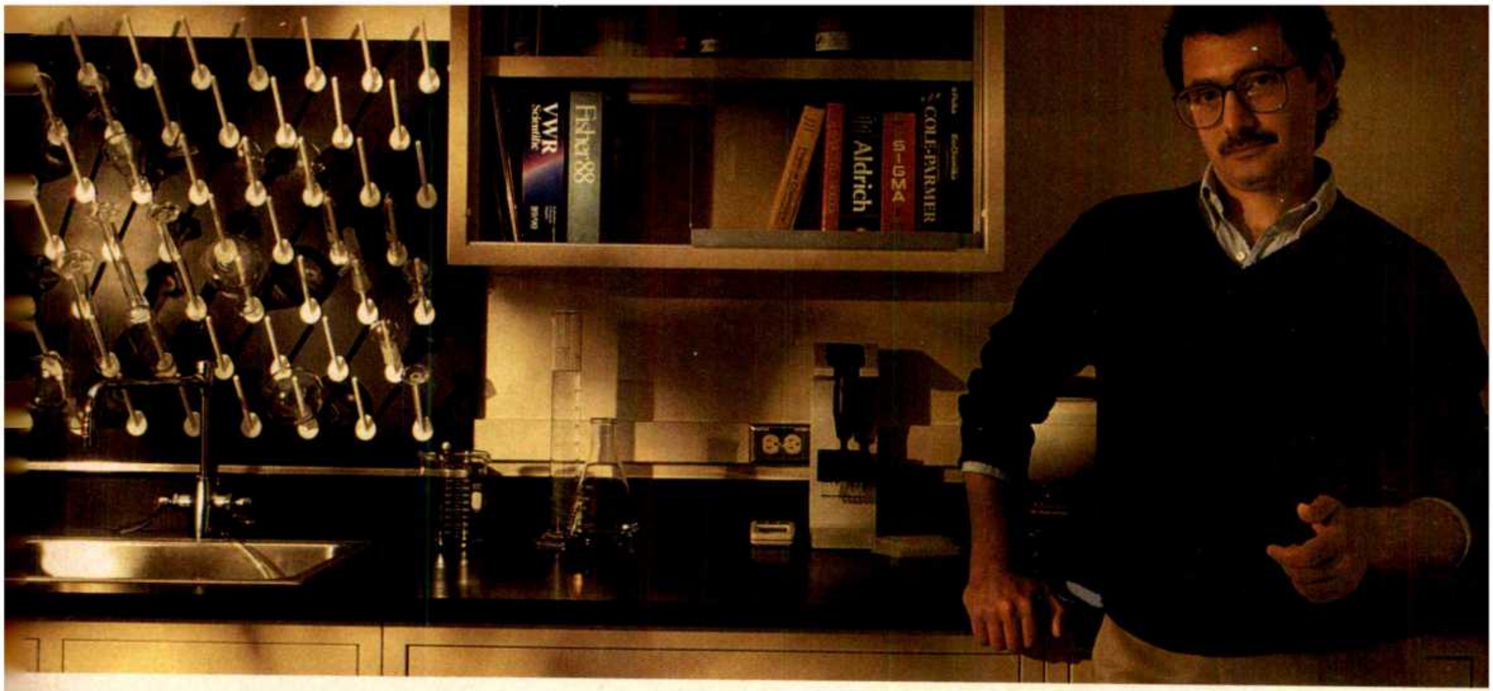

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Activators and targets

Mark Ptashne & Alexander A. F. Gann

Proteins that activate genes are quite disparate in character; in particular, some work 'universally' and others do not. A simple model can accommodate most of the recently published results.

SEVERAL papers recently published in *Nature* and elsewhere address certain details of the mechanism by which eukaryotic transcriptional activators work, and reach conclusions that are in apparent conflict. We shall briefly review the essential conclusions of these papers to see whether they can be reconciled. We begin with an outline of the broad ideas and findings that constitute the background of the new work.

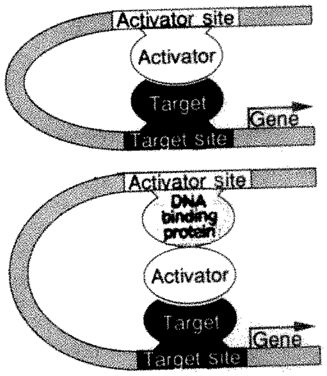


FIG. 1 Gene activation by different kinds of activators. *a*, The activator bears on one peptide a DNA-binding surface and an acidic activating region. The activator, bound at its cognate site, brings to the DNA and/or changes the conformation of a 'universally' conserved target protein that binds near the 5' end of the gene, the intervening DNA looping out to accommodate the reaction. GAL4 is an example of such an activator[1]. *b*, An acidic activating region and a DNA-binding surface are carried on separate molecules, and both molecules are required for activation. Oct1–VP16 is an example[7-11].

According to our current picture, a DNA-bound transcriptional activator interacts with some component of the transcription machinery, bringing it to the DNA and/or changing its conformation on the DNA (ref. 1). This event leads to, or completes, the formation near the transcription start site of a 'pre-initiation complex' that contains several proteins in addition to RNA polymerase itself, including TFIIA, B, D, E and F (ref. 2).

We know of two classes of activators: members of one work 'universally', whereas members of the other work only in certain cells. An example from the first class is the yeast transcriptional activator GAL4: when introduced into any of a wide array of eukaryotic cells, GAL4 activates transcription of a gene bearing GAL4 sites nearby[3-6]. According to the simple model shown in Fig. 1, the key to the universal action of GAL4 is that it bears two functions on a single peptide: a DNA-binding surface and an 'acidic activating region' that interacts with a target protein the relevant surface of which must be conserved among eukaryotes. Each member of the second class of activators, by contrast, bears just one of the two required functions and therefore can work only in cells bearing molecules that provide the missing function. We consider as examples the mammalian DNA-binding protein Oct1 (ref. 7) and the herpes virus protein VP16 (refs 8–11). As shown in Fig. 1, VP16, which bears an acidic activating region, is unable to bind to DNA directly. It can, however, bind to a complex of DNA-bound proteins that includes Oct1, which itself lacks an acidic activating region[12]. Thus VP16 will activate transcription only in cells containing Oct 1, and Oct 1 will activate genes only in cells containing VP16. When VP16 is fused to a DNA-binding fragment of GAL4, the fusion protein works as does GAL4 itself[13]. According to this model, then, the 'non-universal' activators can be understood in the same framework as the 'universal' ones. It may be helpful for what follows to bear in mind that 'activator' is an operational definition, applied to any transcription factor that activates specific genes. Thus the proteins GAL4, Oct1 and VP16 are all called activators, despite their dissimilarities.

The new papers we consider address two questions: first, what is the target protein seen by acidic activating regions? And, second, can we fit into the picture outlined above two additional non-acidic regulators that work only in certain cell types: SP1, a mammalian DNA-binding protein[14], and Ela, an adenovirus protein that does not bind DNA[15]? Both of these proteins activate in mammalian but not in yeast cells, a result that holds for fusion proteins comprising SP1 or the Ela activating region (as defined in mammalian cells) attached to DNA-binding fragments of GAL4 (G. Gill, E. Pascal, R. Tjian and M. P., unpublished; J. Lillie and M. Green, personal communication; ref. 16). We begin by asking what is the target seen by acidic activating regions?

VP16 and TFIID

Stringer *et al.*[17] argue that the activating region of VP16 interacts directly with the protein TFIID. They report that an affinity column bearing VP16 retains a protein — or proteins — that restores activity to a mammalian cell extract that has been depleted of TFIID activity by heat inactivation. Moreover, this column selectively retains yeast TFIID expressed from a cloned gene in bacteria[17]. These results are made more compelling by the claim that there is a good correlation between the strengths of various VP16 mutants, as assayed by their abilities to activate transcription in mammalian cells, and their affinities for TFIID as measured in the column experiment (ref. 17 and C. J. Ingles, M. Shales, D. Cress, S. Triezenberg and J. Greenblatt, personal communication). The result is so important that we look forward to further controls showing, for example, that acidic activators do not interact with other general transcription factors, and that at least some mutants of TFIID that cannot respond to acidic activators in a transcription assay also cannot interact with the acidic activating regions as measured directly.

The idea that TFIID is the target of acidic activating regions is attractive: binding of TFIID is known to precede binding of the other known transcription factors and to 'commit' the template to being transcribed once these have been added[2]. Previous experiments had shown that various activators change the footprint made near the TATA sequence in a partially purified TFIID preparation (a TFIID fraction) from mammalian cells[18,19].

If Stringer *et al.*[17] are right, the phenomenon of universal activation requires that all the TFIID proteins found in various eukaryotes can interact with acidic activating regions. This proposition can be tested now that TFIID genes have been cloned from HeLa cells[20,21], *Drosophila*[22] and *Arabidopsis*[23] as well as yeast[24-26]. As in yeast, each of these organisms evidently has only a single TFIID gene, with the exception of *Arabidopsis*, which has two[23]. These genes are similar throughout their carboxy-terminal 180 amino acids, but differ in their amino termini (Fig. 2). In particular, those of human and *Drosophila* each have a stretch of about 160 amino acids absent from the other molecules. The yeast TFIID has approximately 60 amino acids at its N terminus that bear no similarity to sequences in *Drosophila* or human TFIID.

A missing component

The chromatographic fraction termed TFIID (that which includes the TATA binding protein) has never been purified to homogeneity from extracts of mammalian cells, and it was not unreasonable to expect that this fraction includes more than one component. There is now strong evidence for this. Mammalian TFIID, synthesized in

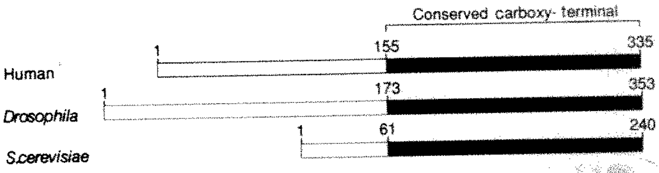


FIG. 2 The products of various cloned TFIID genes[20-26]. The molecules are similar, but not identical, over approximately 180 carboxyl-terminal amino acids. The amino termini bear distinct amino-terminal extensions.

and purified from bacteria, restores basal level transcription to a mammalian extract lacking the TFIID fraction, but does not restore the ability to respond to acidic activators such as GAL4–VP16, or to SP1. In an important control for this result, Peterson *et al.*[20] show that an extract treated with heat to destroy its transcriptional activity can be restored to full inducibility by the addition of cloned human TFIID; this result confirms that heat specifically inactivates TFIID, and that the recombinant protein is functional (ref. 20; R. Tjian, personal communication).

These results strongly suggest that the TFIID fraction of the mammalian cell extracts contains some component or components, in addition to TFIID itself (as now defined by the cloned gene), that is necessary for response to any activator. The fact that TFIID as partially purified from mammalian cells behaves as a larger protein than the cloned TFIID[27] suggests that this new component in the mammalian case at least may be bound to TFIID. Evidently this interaction has some species specificity: addition of excess yeast TFIID to a mammalian or *Drosophila* extract elicits a basal level of transcription that can be activated neither by GAL4-VP16 (R. Tjian, personal communication) nor by SP1[28]. Presumably, in this experiment, the added TFIID competes with the endogenous TFIID for binding to the TATA sequence and, once bound, is unable to respond to activators.

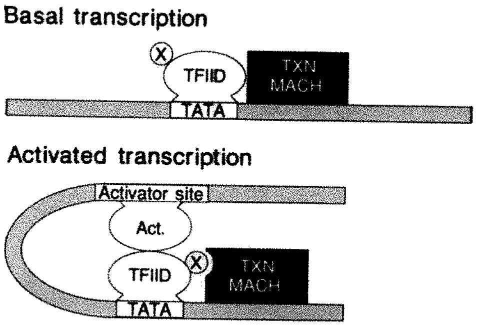
If we accept the results of Stringer *et al.*[17], the extra component(s) required for activation is probably not an intermediary *between* an acidic activating region and TFIID: it is more likely to be a factor required for some step in activation that follows activator-TFIID interaction (see for example Fig. 3). Kelleher *et al.*[29] and Berger *et al.*[30], however, argue to the contrary, proposing the existence of an intermediary between an acidic activating region and TFIID. Their arguments are indirect and rely on "squelching" experiments[1,31] performed *in vitro*. The idea behind "squelching" is that the putative interaction between the activator and the target, as shown in Fig. 1, might also occur off DNA. Thus an activator bearing a strong activating region and present at artificially high concentrations could sequester the target and thereby inhibit, or "squelch", the expression of other genes (Fig. 4). Indeed, expression of a series of GAL4-derived activators at high concentrations in yeast inhibited expression of a heterologous gene, and the degree of inhibition was roughly proportional to the strength of the overexpressed activating regions[31]. Another example is seen with VP16, whose activating region is unusually potent; at high concentrations, VP16 is a particularly strong squelcher in mammalian cells[8,32].

In the squelching experiments of Kelleher *et al.*[29], GAL4-VP16 is added to a yeast extract containing two added genes: one bears GAL4 binding sites upstream and the other dA.dT sequences that presumably bind an endogenous activator. Moderate levels of GAL4-VP16 activate the former as they inhibit the latter (squelching), and high levels of GAL4-VP16 inhibit both (squelching and self-squelching). Kelleher *et al.*[29] then find that this inhibition is not relieved by addition either of TFIID or of polymerase, but is relieved by addition of some other fraction of the yeast extract. The authors surmise that this fraction includes an adapter that bridges TFIID and the acidic activator. Their results are, however, equally consistent (as the authors note in passing) with the idea that the additional component, rather than being an intermediary, is required for some essential step in activation that follows activator-TFIID interaction. Should that third component be limiting, squelching might be relieved by addition of more of that component, but the result would not reveal the species contacted by the activator.

Similar experiments by Berger *et al.*[30] are more difficult to reconcile with those of Stringer *et al.*[17]. Berger *et al.*[30] also demonstrate squelching of a heterologous promoter by GAL4-VP16 in yeast extracts. But if an excess of a short oligonucleotide bearing a single GAL4 binding site is included, activated transcription is squelched but basal level is not. Berger *et al.* interpret these results as follows. In the absence of the competing oligonucleotide, non-specific DNA binding fosters complete squelching; they imagine the formation of a three-component complex consisting of an activator, an intermediary, and TFIID, with both the activator and TFIID making non-specific contacts with DNA. By contrast, when the activator is bound to the short oligonucleotide, the hypothetical intermediary is sequestered but TFIID is not, and thus basal level transcription is undiminished. The authors' interpretation of this experiment would exclude the possibility that VP16 interacts with TFIID, and thus contradicts the results of Stringer *et al.*[17].

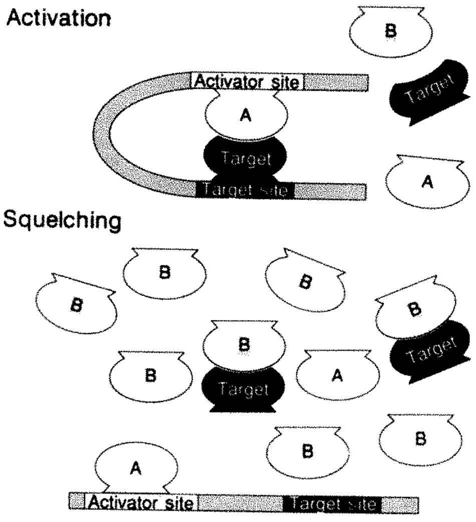
The following experiment, to our knowledge not yet done, might clarify the matter. Squelching by VP16 could be tested in a mammalian cell extract in which cloned TFIID replaces the TFIID fraction; as we have noted, only basal level transcription is observed in such a system. Stringer *et al.*[17] would predict that, even though VP16 cannot activate in this system, it would nevertheless squelch the basal level transcription. Were squelching not observed, the simplest explanation would be that there is an adaptor, as predicted by Berger *et al.*[30], and that that adaptor had been removed as part of the TFIID fraction.

The results described so far (with one exception[30]) are consistent with two propositions, as shown in Fig. 3. First, acidic activators interact directly with TFIID and second, another component(s)


Basal transcription

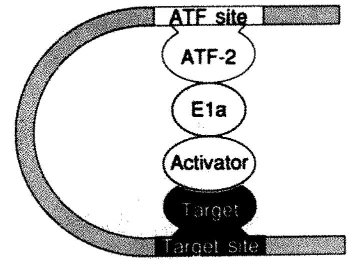
Activated transcription

FIG. 3 An auxiliary protein required for activation. As described in the text, the acidic activator VP16 interacts specifically with cloned TFIID[17]; GAL4-VP16, however, will not activate transcription in a mammalian extract in which the TFIID fraction has been replaced with cloned TFIID[20]. To rationalize these results we imagine a second protein — termed *X* — that is required for TFIID's response to the activator. Protein *X* need not be contacted by the activator, but might nevertheless be bound to TFIID in the absence of the activator (as depicted in the figure). Alternatively, *X* might bind to TFIID only after the activator has caused a conformational change in TFIID. The response of the transcriptional machinery to the activator may involve a change analogous to the transition from a "closed" to an "open" complex elicited by an activator (NTRC) working on *E. coli* RNA polymerase bearing σ54 (refs 37,38). Another interesting analogy is activation of phage T4 late gene expression. The promoters of T4 late genes are recognized by RNA polymerase bearing the T4 encoded σ55. Transcription by this polymerase is activated by a complex of the products of the T4 genes 44, 62 and 45, but only if the polymerase also bears a further protein, gp33, itself not necessary for promoter recognition nor, at least under some conditions, for basal level transcription[39]. The role of gp33 is unknown.


Activation

Squelching

FIG. 4 Squelching. High concentration of activator B sequesters the target protein also seen by activator A, thereby inhibiting activation by A[31]. At very high concentrations activation by A will also be inhibited (self-squelching). Note two features implied by this picture: first, the sequestered protein need not be the target as defined in Fig. 1. Thus, for example, overexpression of E1a presumably sequesters an intermediary protein that itself might bear an activating region that interacts with the target of Fig. 1 (ref. 32). Second, the factor sequestered by the activator need not be a single species and, assuming that any of the peptides in the complex might be limiting, squelching could be relieved by addition of that peptide.



IG. 5 A model for activation by E1a. 1a recognizes, with one surface, a NA-bound ATF-2 (ref. 34), and with nother surface, a protein bearing an cidic activating region. Note that lthough there is evidence suggesting hat E1a contacts an intermediary pro- ein, there is no evidence that that rotein bears an acidic activating egion[32].

must interact with the TFIID-activator complex for a full response to the activator. Before attempts to apply this model to the mammalian activators E1a and SP1, we summarize results of experiments involving nucleosome formation *in vitro* that are consistent with the model.

Workman *et al.*[33] found that when a nucleosome assembly system is added along with the template DNA to a mammalian cell extract, the basal level transcription observed in the absence of the nucleosomes is blocked; presumably nucleosome formation competes with and excludes binding of some transcription factor (for example TFIID). It has now been reported that inclusion of GAL4-VP16 in the initial mix elicits a high level of transcription if the template bears GAL4 binding sites upstream (J. L. Workman, I. C. A. Taylor and R. E. Kingston, manuscript in preparation); evidently the activator helps some transcription factor (such as TFIID) compete with nucleosome formation for DNA binding. When this experiment is repeated, using an extract in which cloned yeast TFIID replaces the TFIID fraction, basal level transcription is observed. Workman and co-workers' interpretation is that, either directly or indirectly, GAL4-VP16 helps yeast TFIID to compete for binding with nucleosomes; only basal level transcription is observed in this case, however, because a subsequent, perhaps species specific, interaction is required for full activation.

E1a and SP1

M. Green and colleagues show convincingly[34] that E1a binds to the DNA-bound protein ATF-2, suggesting that E1a works in a fashion analogous to VP16. But various considerations lead them to suggest that E1a does not bear an acidic activating region, and they interpret experiments involving squelching in mammalian cells as arguing that E1a works by a mechanism that involves a simple extension of model *b* in Fig. 1[32]. They propose that E1a, attached by one surface to DNA-bound ATF-2, in turn attaches by another surface to a third component; that component could bear an acidic activating region (Fig. 5). Their experiments can be summarized as follows: expression of a gene activated by any of several GAL4-derived activators bearing acidic activating regions is squelched by high levels of VP16, but not by high levels of E1a. By contrast, expression of a gene activated by the fusion protein LexA–E1a (which binds to LexA operators) is squelched by high levels of either E1a or VP16. These results are consistent with the idea that E1a is an intermediary that contacts an activator protein that, in turn, contacts the target seen by VP16. Note that, if the argument is correct, the activator protein contacted by E1a cannot be an essential component of the transcription machinery, because high levels of E1a do not squelch VP16-activated genes.

Turning now to SP1, we might imagine three mechanisms for how this non-acidic DNA-binding protein activates. First, it might, like Oct1, bind an activator that bears an acidic activating region that interacts with TFIID as described by Stringer *et al.*[17]. Second, SP1 might, directly or via an intermediary, contact a part of TFIID different from that recognized by the acidic activators. Third, it might contact, directly or indirectly, some component of the transcription machinery other than TFIID. At the moment there is no compelling basis for a choice between these models. Pugh *et al.*[28] show that mammalian recombinant TFIID is unable to substitute for the TFIID fraction in supporting a response to SP1, a result they interpret as indicating the need for an intermediary between SP1 and the TFIID polypeptide. But as we have noted, the same result holds for acidic activators and, assuming that Stringer *et al.*[17] are right, the missing component(s) could be required for activation without interacting with the activating region (Fig. 3). The sequences of the various cloned TFIIDs could hint at a version of the second model: SP1, which does not activate in yeast, might recognize the amino-terminal extension found on human and *Drosophila* TFIID that is missing from yeast TFIID. Unfortunately, deletion of that part of human TFIID, which abolishes the response to SP1, also abolishes the response to an acidic activator[20], and so the question remains undecided.

Conclusion

It is possible (*pace* Berger *et al.*[30]) that the following simple model holds: all activators exert their effects by interacting with TFIID: acidic activators work directly, and hence universally, and others (Oct1, SP1, E1a, for example) do so via intermediaries bearing acidic activating regions. Even if the experiment of Stringer *et al.*[17] were to turn out to be misleading, and acidic activators were to recognize some target other than TFIID, it would still be possible that all activators work through that target. But as the models for the possible action of SP1 suggest, there are other, more elaborate, possibilities. In any event, at least one aspect of the simple model is puzzling: it is now well established that heterologous acidic activators can work synergistically to activate transcription, and recent experiments support the idea that their effect is caused by several acidic activating regions simultaneously touching the target[35,36]. If TFIID is indeed the target of acidic activators, we will have to explain how these multiple activating regions can simultaneously touch the rather small (M_r 38,000) TFIID protein.

Note added in proof: Cheng Kao *et al.* (*Science* **248**, 1646–1650, 1990) also report the cloning of human TFIID. They find that when their cloned mammalian TFIID, or cloned yeast TFIID, replaces the TFIID fraction in a mammalian extract, transcription is activated by a protein (presumably SP1) that binds to GC-rich sequences near the gene. This result is in striking contrast to results cited in the text[20,28]. ☐

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Oxygen-defect flux pinning, anomalous magnetization and intra-grain granularity in $YBa_2Cu_3O_{7-\delta}$

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The anomalous magnetization characteristic, intra-grain granularity and the magnitude of the flux pinning in single crystals of $YBa_2Cu_3O_{7-\delta}$ are shown to be intimately linked. As the nominal oxygen deficiency δ decreases towards zero, the flux pinning declines and the crystals lose their explicitly granular signature. As δ is seldom specifically controlled at levels below ~0.05 in even the most carefully made materials, small oxygen deficiencies may contribute significantly to both the flux pinning and the weak-link properties.

THE flux pinning and granular properties of high-T_c superconductors continue to be a perplexing problem. Crystallographic grain boundaries are strongly implicated as the decoupling sites in these materials[1] and thus single crystals should be important for the study of the basic pinning mechanisms. It has been shown, however, that single crystals separated from bulk polycrystalline samples can also become granular (that is, internally subdivided)[2,3]. Also, a study of a large single crystal of $YBa_2Cu_3O_{7-\delta}$ with a low T_c of 82 K showed it to be granular[4]. On the other hand, it has also been reported that individual crystals are fully coupled[5,6]. Thus there is no general agreement on whether intra-grain weak links are generally present and measurements on 'good' single crystals over a wide range of temperature and field are needed to resolve this important issue. Here we describe such measurements.

Magnetization characteristics

We used two crystals of $YBa_2Cu_3O_{7-\delta}$ grown from CuO-rich flux[7,8] and one grown by a liquid-phase process[9]. Details are given in Table 1. The first crystal (FG1) was selected because of its high T_c, the sharpness of its a.c. inductive transition and its complete Meissner flux exclusion in fields <0.1 mT, which indicate that this crystal should be considered 'good' by present standards. Our characterization confirmed the very sharp transition in the zero-field a.c. susceptibility (T_c midpoint 93.5 K, ΔT_c (10%, 90% signal) <1 K). But when weak d.c. magnetic fields were applied, the transition was found to occur over several degrees. In all but the weakest magnetic fields (~1 mT), we observed smeared double transitions, indicative of granularity. Striking effects were also seen in magnetization measurements made with a vibrating-sample magnetometer. Figure 1 shows the magnetization loop at 70 K (for an applied field H_a parallel to the c axis, as was the case for all measurements reported here); an anomalous and large second peak was observed at ~4.5 T, the magnitude of the second peak being about twice the zero-field peak. In Fig. 2, the magnetization data for the range 10–80 K and up to ~8 T is reduced to J_c using the standard expression $J_c = \Delta M/(2r)$, where ΔM is the width of the hysteresis loop and r the effective crystal radius ($r = \frac{3}{2}l_1(1 - l_1/3l_2)$, where l_1 is the shorter dimension, perpendicular to the field). The double peak is observed from 30–80 K, although the anomaly is particularly evident above 50 K. Observation of the second peak requires measurements over a wide range of T and H and therefore the second peak is frequently not seen. The minimum in M or J_c, however, is generally visible but almost invariably ignored[10-13]. Thus, the effect is very general in twinned and untwinned crystals, which are normally accepted as being 'good', as well as in samples recognized to be of lower quality[4]. Further insight is provided by the data of Fig. 3, which shows the magnetization at fields of 0, 1 and 4 T at 80 K for three pieces of the crystal (FG1) after subdivision. At low fields (0–1 T), M is proportional to size, as is expected for a coupled crystal, whereas at higher fields (4 T), well above the second peak, M is independent of r. The latter behaviour is characteristic of a granular crystal. At low temperatures, however, M (0–4 T) was always proportional to size[14].

Oxygen deficiency

One of the variables that these results stimulated us to explore was the effect of small oxygen deficiencies δ on the superconducting properties. Oxygen diffusion coefficients in $YBa_2Cu_3O_{7-\delta}$ are rather uncertain[15,16] and the oxygen content of single crystals, weighing only a few hundred micrograms, cannot be explicitly checked by the techniques applicable for bulk samples. A common oxygenation applied to flux-grown crystals is 240 h at 420 °C[7,8] for which the equilibrium value of δ would be ~0.04 (ref. 17). In zero-field measurements[18], T_c has been shown to be insensitive to δ for $\delta \leq 0.1$, but this result has not been checked in a non-zero field. Thus the variation of the magnetization properties with oxygenation was the focus of the second part of the experiment. We used two new crystals—one, FG2, was grown in a similar manner to the previous

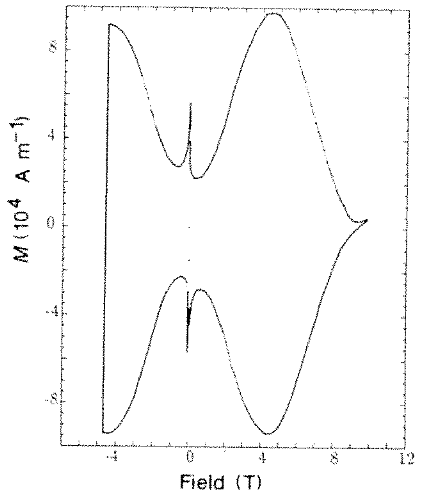

FIG. 1 Magnetization loop of flux-grown crystal 1 at 70 K.

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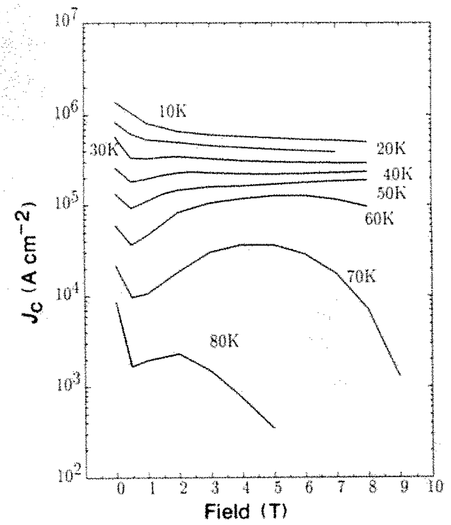

FIG. 2 Critical current density of flux-grown crystal 1 derived from the magnetization loops using the sample size as the scaling radius.

crystal[7,8]. Like FG1, it was extensively twinned but had no observable second phase. The second sample was made by a modified liquid-phase processing (LPP) route[9]. A highly textured, multi-domain sample composed of about 15 crystals stacked on top of one another was cut from a larger polycrystalline mass. The microstructure of the individual grains contained ~20% of Y_2BaCuO_5 ('211') phase of no obvious preferred orientation. The boundaries between the crystallites were *a*-*b* planes, for which the misorientation was very small (the 006-plane rocking curve had a full width at half maximum of ~0.8°). The *a* and *b* directions of neighbouring layers in the crystal also seemed to be very well aligned. Thus, in the direction for which we made measurements (applied field parallel to the *c* axis), there are no crystallographic grain-boundary barriers to current flow and from an electromagnetic point of view the sample can be considered to be a 'single crystal'.

Initially we equilibrated the specimens at 600 °C for 100 h in 1 atm of flowing oxygen, followed by 100 h at 525 °C. After this treatment, T_c was $\leq$80 K. Here we report data taken after subsequent oxygenation, for which T_c was $\geq$90 K. Derived J_c values for the crystals are shown in Fig. 4. We note several features—first, in both samples the derived J_c decreases with further oxygenation. Thus decreasing δ does reduce the flux pinning. Second, as the oxygenation improves, the magnetization anomaly tends to disappear. This was particularly true for the LPP crystal, for which the anomaly was still visible after the shortest oxygenation (100 h at 450 °C). The anomaly of the FG crystal, however, did not disappear even after >900 h of oxygenation at $\leq$450 °C. Third, the J_c of the flux-grown second-phase-free single crystal exceeds that of the LPP sample, which contains ~20% of 211 phase, over a considerable range of the magnetic field, whether measured at high (70 K) or low (10 K) temperature. It is therefore unlikely that the large (2-10 μm diameter) second-phase particles of the LPP sample are contributing significantly to the flux pinning.

The intra-grain granularity was again examined by subdividing the crystals after their penultimate oxygenation (200 h with slow cooling from 400 to 350 °C). The anomaly for the FG2 crystal was still very clear—J_c rises to a distinct peak at ~2 T at 70 K, but at 50 K the maximum was smeared out in the range 2-8 T. For the LPP crystal, however, no such anomaly was observed after the oxygenation exceeded 100 h at 450 °C. The size-scaling results of Fig. 5 for the LPP and FG2 crystals are also very different. For the LPP crystal, $\Delta M(H)$ is proportional to r at both 10 K and 70 K, whereas for the FG2 crystal, the behaviour is more complex. At 10 K, no anomaly is seen ($M \propto r$ for $H = 1$ T, 4 T). This is also the case at 70 K for a magnetic field of 1 T, which lies below the $\Delta M(H)$ peak at that temperature. At 70 K and for magnetic fields of 4 T and 6 T, M becomes independent of size. Thus, evidently granular behaviour is visible for crystal FG2 beyond the second peak in the magnetization curve, just as it was for FG1 (Fig. 3).

TABLE 1 Details of crystals

Crystal	Source	Size*	Comments
FG1	IBM	~0.85 mm radius × 42 μm†	Flux grown, single crystal
FG2	NIST‡	1.16 × 0.90 mm × 62 μm†	Flux grown, single crystal
LPP	UW§	2.2 × 1.8 mm × 65 μm†	Liquid-phase process, multi-domain crystal

* Before breaking.
† Thickness in the *c* direction.
‡ National Institute of Science and Technology.
§ University of Wisconsin.

Discussion

These data are all consistent with the proposal that oxygen defects act as significant pinning centres. In the absence of a magnetic field, proximity-effect coupling between the oxygen-rich and oxygen-deficient regions maintains a sharp zero-field T_c value at a high value[18,19]. Oxygen-deficient regions should have a markedly lower $T_c(H)$ in a non-zero field, however, and can broaden the T_c transition as the field increases. The elementary pinning force per defect ($\sim\frac{1}{2}\mu_0\Delta H_c^2 V_d$ where ΔH_c is the critical-field difference between matrix and defect and V_d is the fluxon core volume intercepted by the defect) increases as the field suppresses superconductivity in the oxygen-deficient region, causing the magnetization to rise steeply beyond the minimum in the M-H curve (Fig. 1). Similar, but less marked behaviour, has been observed for Sn precipitates in a Pb-In-Sn alloy[20]. Here, however, we have shown that the flux pinning and the granular properties are linked. Evidently there are enough oxygen-deficient regions[21-23] to link up and provide internal barriers, rendering the crystal granular. The steep fall in the magnetization hysteresis (Fig. 1) beyond the second peak is then at least partly due to a progressive reduction in the scaling radius as the field increases. Plots of the derived J_c (such as Figs 2, 4) therefore become unreliable when the scaling radius is ill defined, as it evidently is at higher fields for the flux-grown crystals. As expected, M increases at higher fields for specimens with larger oxygen contents, reflecting the better size scaling.

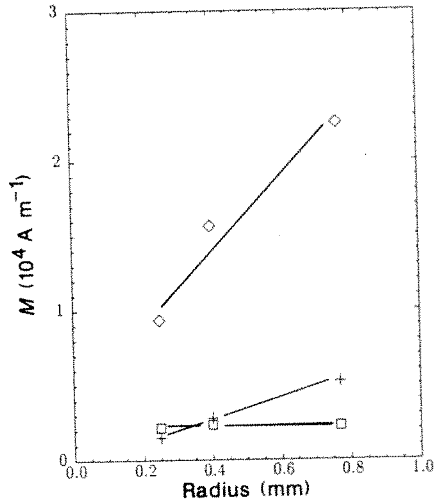

FIG. 3 Magnetization hysteresis plotted against radius for flux-grown crystal 1 at 80 K. ◇, 0 T; +, 1 T; □, 4 T.

An important issue raised by our data is the large magnitude of J_c in the crystals. In optimized NbTi with 47 wt% Ti, where very strong flux pinning exists, 20 vol% of nanometre-scale α-Ti precipitation[24,25] leads to J_c values of the order of 10^{-2} of the depairing current density, J_d, roughly the same as for these crystals. But, as already discussed, the second-phase precipitates of the LPP crystal do not provide strong pinning. Twins must also be considered as pinning sites. Although there is abundant evidence to show that twins pin flux[10-12], there is no consensus on the relative strength of twin pinning compared to other sources of pinning. A recent study[12] of the same crystal in both the twinned and untwinned state showed that the residual pinning of the untwinned state was, in general, significantly larger than the pinning produced by the twins (except at 10 K for H parallel to the a–b plane)[12]. These studies are consistent with the conclusion that there is strong residual pinning, whatever the twin or second-phase density. We therefore conclude that small oxygen deficiencies have a significant pinning role. The average distance d between unordered O vacancies is $\sim a/\sqrt{\delta}$ where a is the lattice parameter of the basal plane; for $\delta = 0.05$, $d \approx \xi_{ab}$, an almost ideal pinning situation. Unfortunately, oxygen deficiencies are not useful pinning centres, because their deleterious granular behaviour outweighs the benefits of the extra pinning that they provide.

Control of oxygen deficiency at the very low levels studied here is very difficult. As mentioned above, the single crystals are small and their oxygen content cannot be verified by thermogravimetric analysis (TGA). Equilibrium values of δ for 1 atm O_2 lie in the range 0.02–0.04 at 350–450 °C (ref. 17). After the last treatment given (480 h at 375 °C), the LPP crystal J_c values were virtually unchanged, whereas those for the flux-grown crystal were still decreasing. Even for polycrystals, where TGA is quite feasible, explicit checks of the degree of oxygenation are seldom made and measurement of the magnetization curve may provide the best guide to the oxygen content of the sample. Different defect densities (such as the difference between flux-grown and LPP crystals) must certainly be expected to affect the oxygenation kinetics.

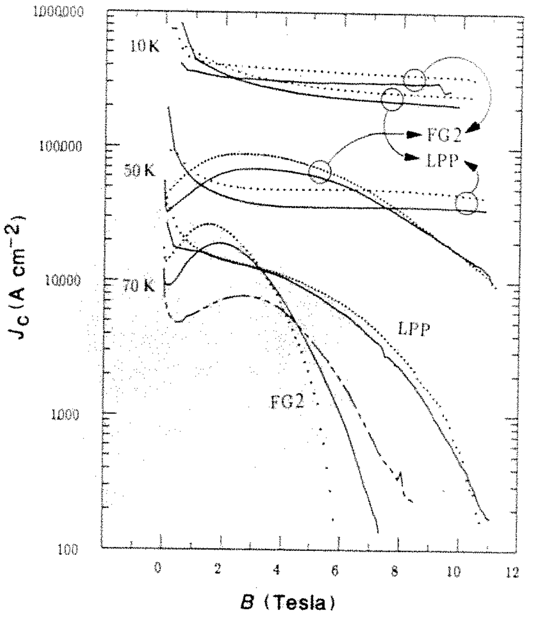

FIG. 4 Critical current density at various temperatures derived from the magnetization loops using the sample size as the scaling radius. Dotted lines correspond to low-temperature oxygenations of 300 h at 450 °C (LPP) and 200 h at 450 °C (FG2) and solid lines correspond to a further oxygenation of 200 h with slow cooling from 400 to 350 °C. The dashed curve corresponds to a further oxygenation of 480 h at 375 °C for the FG2 sample.

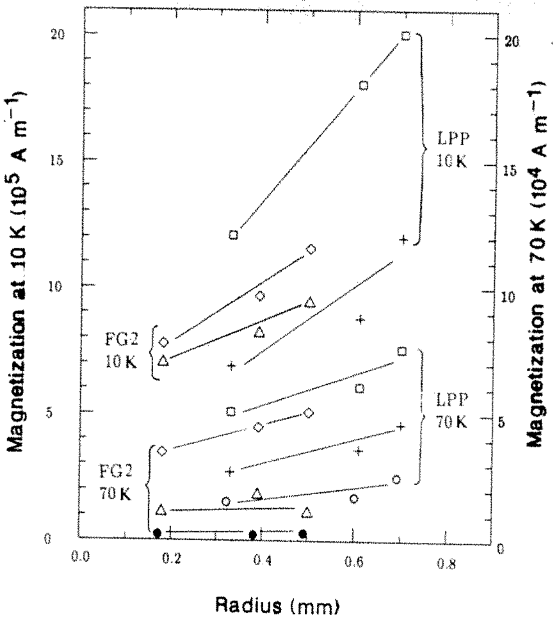

FIG. 5 Magnetization hysteresis measured at 10 K and 70 K at various fields for both the LPP and FG crystals. The radius was varied by subdividing the crystals. The oxygen condition was 200 h with slow cooling from 400 to 350 °C. LPP: □, 1 T; +, 4 T; ○, 6 T. FG2: ◇, 1 T; △, 4 T; ●, 6 T.

There are two final issues to discuss. The first concerns another aspect of the size-scaling results. In Fig. 5 it can be seen that, even in the regimes where $M \propto r$, none of the extrapolations go through the origin, as would be expected for fully coupled crystals. This suggests that there is some residual granularity for all conditions studied, consistent with several other studies[2-4]. It is noticeable, however, that the data for the LPP crystal, which show no anomaly, extrapolate to lower values of ΔM than is the case for the FG2 crystal, which does show an anomaly, and this is consistent with the through-origin size-scaling results on quench-melt-grown (QMG) crystals obtained by Murakami *et al.*[6] in the range 0–1 T at 77 K. The second-phase and low-angle grain boundaries of LPP and QMG crystals should certainly enhance their oxygenation as compared with the more perfect FG crystals. Thus several granular properties that are sensitive to the precise defect structure of the crystal may exist[4]. The second point concerns the generality of our results. Although the nature of the samples is very different—two are second-phase-free single crystals grown by a flux route in different laboratories, whereas the third is a highly textured crystal containing some 20% of the 211 phase—all three samples exhibited qualitatively the same behaviour. Indeed, anomalous magnetization curves are very commonly observed in 'good' single crystals made in different ways[4,11-13] and in polycrystalline samples[4,21]. In fact, as discussed above, it is reasonable that samples generally have small oxygen deficiencies. At the present stage of high-T_c single-crystal studies, there are still many unknown material parameters and the effects reported here are clearly evident in precisely the types of crystals on which much of the basic physics of the compounds is being experimentally studied.

In summary, field-induced granular behaviour has been observed in three crystals of $YBa_2Cu_3O_{7-\delta}$. We have varied the oxygenation conditions of two crystals in such a way as to make their δ values small. Decreasing δ tends to eliminate the anomalous double-peaked magnetization curve generally found in single crystals of $YBa_2Cu_3O_{7-\delta}$ compounds. Further, decreasing δ decreases the flux pinning in the crystals and granular behaviour is seen above the second peak in the magnetization curve. We conclude that all three features, the anomalous magnetization curve, the flux pinning and the granular behaviour are related to the oxygen deficiency, even when δ is of the order of 0.05. Explicit verification that these anomalies are absent is important for understanding the flux pinning, and the granular and reversible properties of the $YBa_2Cu_3O_{7-\delta}$ compounds. In particular we note that practical applications of these materials will require elimination of both the intra-grain weak links identified here, as well as the more generally recognized intergranular weak links. ☐

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Movement of internalized ligand–receptor complexes along a continuous endosomal reticulum

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Complexes of cell-surface receptors and their ligands are commonly internalized by endocytosis and enter a prelysosomal endosomal pathway for further processing. Fluorescence microscopy and video recording of living cells to trace the passage of ligand–receptor complexes has identified the endosomal compartment as an extensive network of tubular cisternae. Endocytosed material entering this reticulum enters discrete swellings, identified as multivesicular bodies by electron microscopy, which move along the reticulum towards the pericentriolar area.

THE acidic endosomal compartment has a central role in the receptor-mediated uptake and processing of a wide range of metabolites (such as cholesterol) and pathogens, and in the recycling of receptor–ligand complexes[1-3]. The membrane boundary of the endocytotic pathway is also probably involved in the transduction of mitogenic signals. Defining the limits of this system and identifying its regulatory mechanisms are therefore of considerable current interest in cell biology.

The endosome is a difficult organelle to isolate by conventional methods of subcellular fractionation because it lacks a characteristic buoyant density and is highly pleiomorphic. It has been studied most successfully by microscopical methods and some of its component parts have been described in detail[4-6]. However, the overall form of the organelle and the way in which its diverse functions are regulated remain unclear.

We have applied various microscopical methods to define the form of the endocytotic pathway in living cells. Here we describe confocal microscopy and low light video recording of living epidermoid carcinoma cells (Hep-2) to follow the movement of fluorescent tracers through the endosomal compartment. Contrary to expectation we have found an extensive tubular reticulum with expanded swellings along its length. Electron microscopy shows that these structures are typical multivesicular bodies, endosomal elements which have been identified previously in fixed cells and which hitherto had been thought to be free vacuoles.

Identification of an endosomal reticulum

The iron-carrying protein transferrin enters cells by receptor-mediated endocytosis. Transferrin labelled with the dye Texas

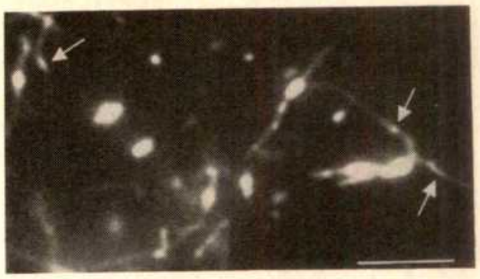

FIG. 1 Cisternal elements in peripheral cytoplasm of Hep-2 carcinoma cells. The fine branching cisternae have varicosities of varying sizes along them. Arrows indicate moving flows of tracer which on video show direction and rate of movement. Cells were grown in Dulbecco's MEM plus 5% fetal calf serum in sparse culture as described previously for A431 cells[7]. They were incubated in subsaturating (10^{-9} M) concentrations of TF-TXR for 60 min or more at 37 °C. Image obtained with a Hamamatsu C 2400-08 SIT camera. Scale bar, 5 μm.

red (TF-TXR) was used as tracer for the endosomal pathway in living cells. To localize internalized receptors, anti-transferrin receptor antibodies (ATR) tagged either with the fluorescent label FITC or (for electron microscopy) with gold, were used as probes.

The endosomal system was loaded to steady state[7] by incubating Hep-2 cells for 60 min or more at 37 °C with subsaturating concentrations of TF-TXR which binds to transferrin receptors. Confocal and low light video recording with an SIT camera then identified and followed the tracer as it moved through the cells. Confocal microscopy was used to compare the distribution of fluorescent- or gold-labelled anti-receptor antibodies.

Previous studies on fixed cells have shown internalized transferrin tracers to be distributed throughout the cell within irregularly shaped but often elongated elements[7,8]. Using the SIT camera to follow the movement of the fluorescent tracers within living cells it was clear that many of these elements were interconnected by fine tubular cisternae (Fig. 1). Even though the system was loaded to steady state the tracer was often seen to move as a concentrated bolus through these tubules. As a rule the moving bolus did not alter the dimension of the tubules and by measuring the displacement of these concentrations with time, rates of movement in the range 0.5 to 1.0 μm s^{-1} were obtained. The tubules often branched to form interconnected networks (Fig. 2) and within these networks concentrated flows of tracer were seen to break into two separate streams as the moved through a branch point. This pattern of behaviour wa displayed by both TF-TXR and ATR-FITC tracers and as pulse chase experiments using radiolabelled TF-TXR show the sam kinetics of uptake and recycling as [^{125}I]transferrin (data no shown) we believe that the system outlined is endosomal and separate from the tubular lysosomal elements described in previous reports[9,10].

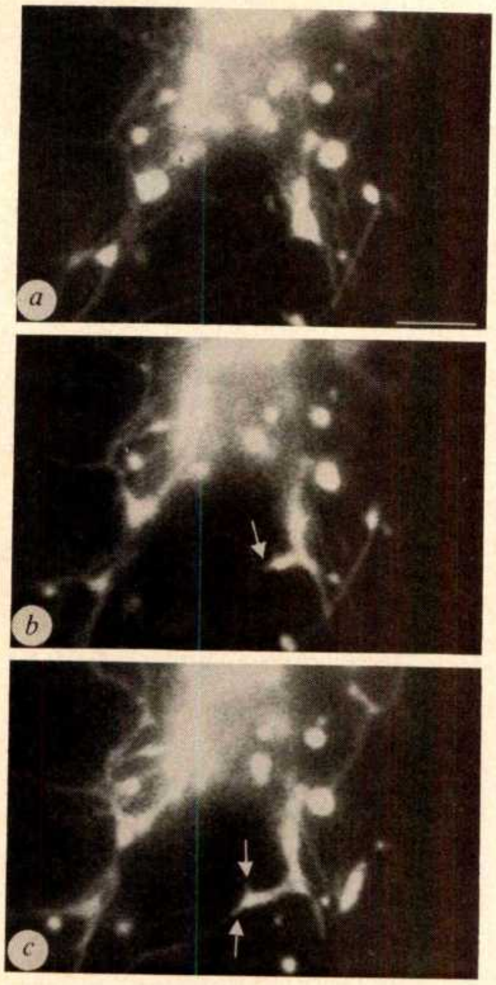

FIG. 2 Endosomal elements in the pericentriolar area loaded with TF-TXR show a complex of bulbous varicosities interconnected by fine branching tubular cisternae. *b* and *c* taken from video 40 s apart. Arrows indicate flow of tracer moving into bifurcating branches of cisterna. Preparation and video recording using SIT camera as in Fig. 1. Scale bar, 5 μm.

Brightly fluorescent swellings or 'varicosities' with diameters of between 0.1 and 0.6 μm occurred along the tubular cisternae at irregular intervals (Fig. 1). These varicosities also moved along the tubules, a movement which could be readily distinguished from the moving concentrations of tracer described above because the localized dilations themselves were displaced. Also, as described in detail below, varicosities move much more slowly.

The tubular reticulum could be found in all parts of the cell but did not seem to form a single, continuous organelle. The longer cisternae (<5 μM) were frequently orientated towards the centrioles and were sometimes seen to extend from the peripheral cytoplasm, just below the plasma membrane, down into the pericentriolar area. Tracer moving within these tubules did not seem to follow any preferred direction (such as towards the nucleus).

The finer tubules in the reticulum were very susceptible to breakdown. Ultraviolet illumination for more than a few minutes, low temperature (20 °C), increased pH or other suboptimal culture conditions caused it to break up into short tubulo-vesicular elements. These fragmented elements usually exhibited a hesitant, saltatory movement[11].

EM of transferrin–receptor complexes

The fate of transferrin–receptor complexes was also followed by electron microscopy. Cells were labelled with gold-anti-receptor antibody. In plastic sections, as described previously[7,12], gold-labelled elements in the peripheral cytoplasm appeared as tubulo-vesicles 30–50 nm in diameter and vacuoles 0.3–0.5 μm in diameter. Only in thick 1-μm sections was there any indication of a cisternal network.

Many of the 0.3–0.5 μm vacuoles lying immediately below the plasma membrane (Fig. 3*a*, *b*) displayed a distinctive cup-shaped form. The origin of these structures, which after short incubations with tracer were the first to be labelled, was not apparent. In vacuoles which become labelled after the cup-shaped structures there were usually two or three internal vesicles within the lumen. Most of the vesicles within these multivesicular bodies (MVBs) were 30–50 nm in diameter and at this time they were closely associated with the perimeter membrane (Fig. 3*d*). Frequently, there was also a larger (~0.3 μm diameter), centrally placed vacuole.

The ATR-gold label was associated exclusively with the inner face of the perimeter membrane (Fig. 3*c*–*e*), and does not enter the internal vesicles.

Processing of EGF–receptor complexes

The endocytotic pathway transfers some ligand–receptor complexes to the lysosome while recycling others to the plasma membrane and so must contain a mechanism for sorting receptor populations. To shed light on this problem we have labelled epidermal growth factor (EGF)–receptor complexes[13] and followed their fate in the endosomal pathway. Internalization of EGF receptors is induced by the binding of EGF. To correlate light and electron microscopical preparations directly we have used an antibody (108) which binds to EGF receptors without inhibiting EGF binding or inducing internalization[14,15]. By coupling this 108 antibody to fluorescent (FITC) or gold labels the trafficking of EGF–receptor complexes could be followed by both light and electron microscopy. In double-label experiments (Fig. 4) we were able to show that fluorescent and gold labels colocalized.

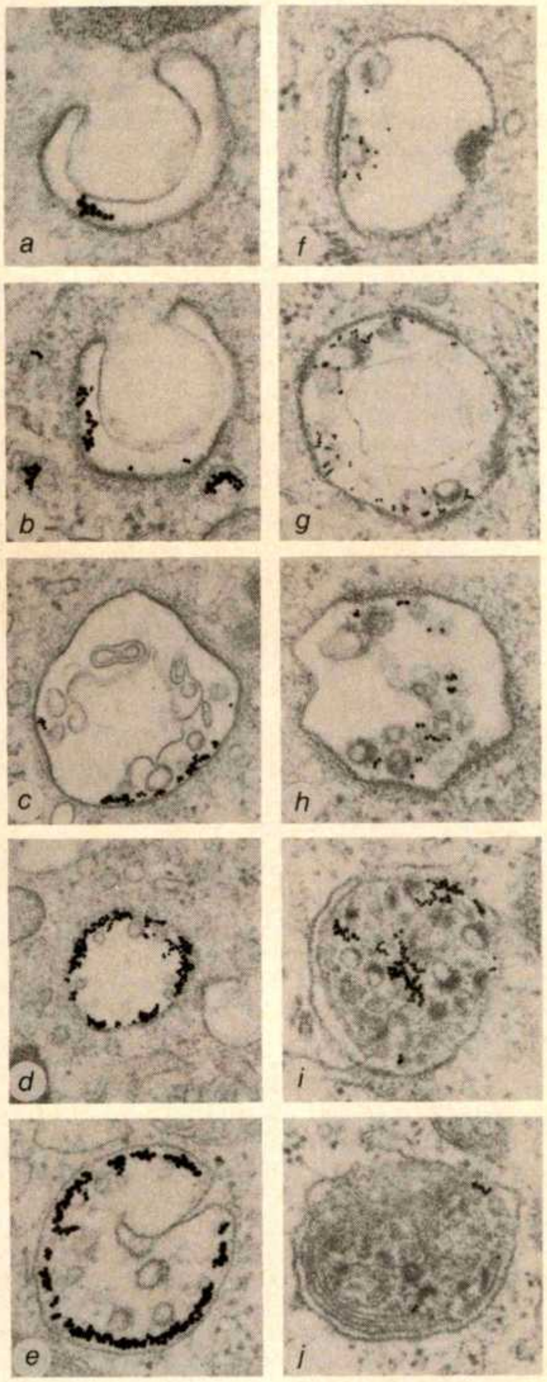

FIG. 3 Electron micrographs showing endosomal elements varying in size between $0.3\,\mu$m and $2.0\,\mu$m in diameter, containing ATR-gold (*a–e*) or gold-labelled anti-EGF receptor antibody (*f–j*). *a, b,* Earliest labelled cup-shaped vacuoles; *c,* multivesicular body with label on its perimeter membrane; *d, e,* multivesicular bodies from the pericentriolar area containing internal vesicles, the gold label remaining on the perimeter membrane; *f,* earliest stage of 0.3–$0.5\,\mu$m diameter vacuole found. EGF receptor label is on an internal vesicle and there is a local invagination of the perimeter membrane on the right hand side of the vacuole; *g,* multi-vesicular body similar to *b* but cut equatorial to the cup-shaped invagination and showing EGF receptor label on small internal vesicles; *h, i,* multivesicular bodies with increasing numbers of EGF receptor-labelled internal vesicles; *j,* labelled lysosome-like structure from the periocentriolar area. Cells were prepared as in Fig. 1 and incubated with gold-antibody conjugates at 5 °C for 30 min, warmed and incubated with 20 nM EGF for 3–30 min at 37 °C before being fixed and prepared for electron microscopy. All reagents and procedures as described previously[7]. Scale bars, 100 nm.

Initially, EGF caused 108-FITC to internalize into elements 0.1–$0.5\,\mu$m in diameter distributed throughout the peripheral cytoplasm, but with time (≤ 30 min) the label concentrated in the pericentriolar area. In cells incubated to steady state with TF-TXR the EGF-receptor could be detected within the varicosities of the transferrin-labelled cisternae. In EGF-stimulated cells the tubules outlined by TF-TXR were greatly elongated and there seemed to be a significant increase in the number of varicosities throughout the cell. Close examination with the SIT camera showed that many of the varicosities were, themselves, moving along the tubules. They moved at a rate of approximately $0.05\,\mu\mathrm{m\,s}^{-1}$ and seemed to retard the flow of the faster moving concentrations of tracer (described above) because the tracer could often be seen backed up in the tubules behind them. An example of varicosity movement is shown in Fig. 5. In this sequence a varicosity is moving down one side of a U-shaped tubule and tracer is filling the tubule to its rear. When the varicosity reaches a branchpoint in the reticulum the transferrin tracer can by-pass the restriction caused by the varicosity and flow rapidly away into ill defined side branches (Fig. 5*c*). As this occurs, the length of the transferrin tracer in the tubule behind the varicosity is reduced.

In the electron microscope the distribution of 108-gold was the same as that shown previously for internalized EGF–receptor using cryosection immunocytochemistry[6]. These results suggest therefore, that receptor sorting occurs in the MVBs/varicosities, as EGF–receptor complexes, but not transferrin-receptor complexes, enter the internal vesicles of the MVBs and are then transported towards the pericentriolar area. At the earliest time points after EGF stimulation 108-gold complexes were present on the perimeter membranes of 0.3–$0.5\,\mu$m vacuoles but were not seen in the cup-shaped elements. From 3 min onwards they were found increasingly on the internal vesicles of MVBs (Figs 3*g, h, i*). From 20 min after EGF stimulation MVBs packed with 108-gold labelled internal vesicles began to accumulate in the pericentriolar area (Fig. 3*i*). From 45 min onwards the gold-labelled vacuoles contained fewer vesicles but included a variety of membrane whorls and myelin figures typical of lysosomes (Fig. 3*j*).

Transport along the endocytotic pathway

Our studies show that the endocytotic pathway includes a branching reticulum with tubular elements up to several micrometres long. The discovery that the endocytotic pathway is more continuous than is apparent in fixed preparations does not alter the consensus view of the organelle as a membrane-bound

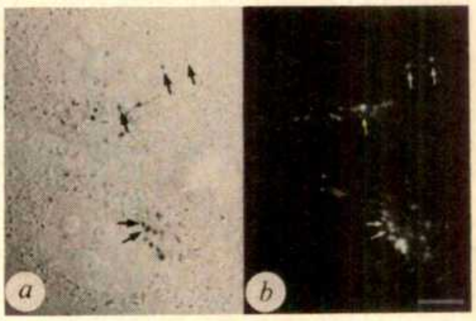

FIG. 4 Colocalization of (*a*) gold-labelled EGF receptor (with antibody 108-gold) and (*b*) fluorescently labelled EGF receptor (with antibody 108-FITC). Arrows indicate the same discrete compartments labelled in both fields. Cells incubated with 108-FITC and 108-gold for 60 min at 4 °C, then stimulated with EGF. Scale bar, $10\,\mu$m.

processing compartment of low pH. It does suggest, however, that many of the molecular interactions that underlie the selective trafficking of membrane proteins and signal transduction should now be looked for within the planes of single membrane boundaries. Separate vesicular compartments and selective vesicle fusions may not be as important as recent studies on cell-free systems suggest[16]. Moreover, alternative ways of generating microenvironments of lowered pH may now need to be considered.

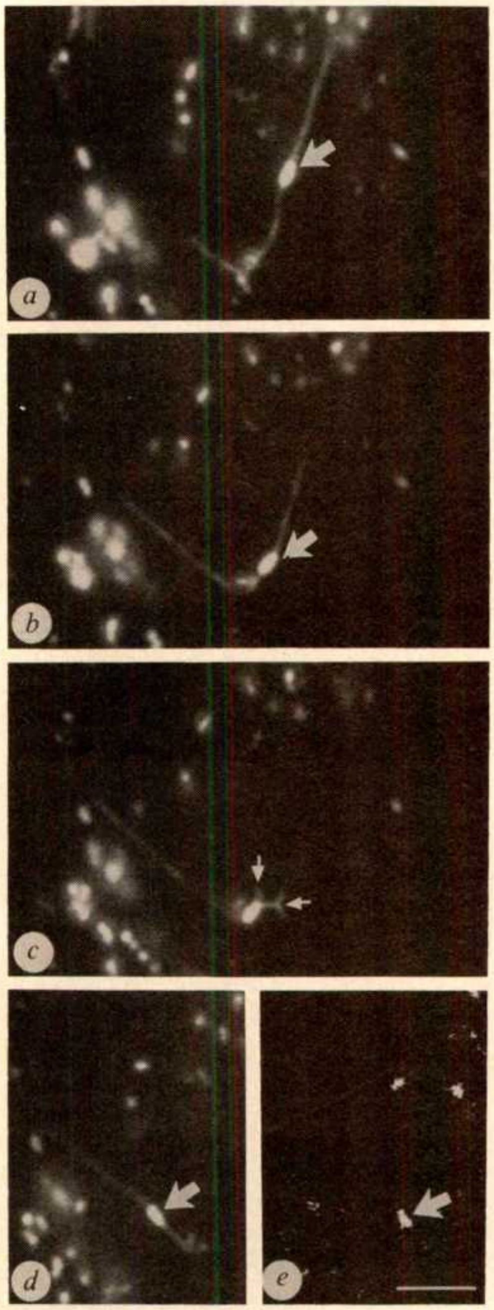

FIG. 5 Branching tubular cisterna showing multivesicular body/varicosity moving along it. Video frames taken from a sequence of images 30 s apart. In *a–b* the varicosity (arrowed) is moving down the right arm of the cisterna. At *c–d* it moves around the bend and up the left arm. Small arrows in *c* indicate side branches into which the tracer TF-TXR flows once the varicosity moves beyond them. *e*, FITC image showing distribution of EGF receptor (labelled with 108-FITC). Arrow indicates the same varicosity as in *d*. Cells cultured as in Fig. 1 then incubated at 5 °C for 30 min with 108-FITC and 20 nM EGF, then rinsed and incubated for 30 min with TF-TXR at 37 °C. Scale bar, 5 μm.

Processing of ligand–receptor complexes

Our finding that the varicosities of the endosomal reticulum seen in living epithelial cells are MVBs shows that the lumin of these vacuolar structures are confluent with the cisternae o the reticulum. Our observations further suggest that trafficking proteins are carried to the MVBs through the tubules. Many previous studies have shown that MVBs with tubulo-vesicula extensions contain ligand-receptor complexes and fluid-phase tracers[10,17]. There is also good immunocytochemical evidence to show that vacuolar structures with tubular arms (CURL) are an important site of ligand-receptor dissociation[4]. Detailed models of the way in which receptors might be removed from MVBs into their cisternal extensions have been proposed[18] bu the possibility that ligand-receptor complexes are being delivered to the MVB by way of a reticulum has not been considered. From our observations it now seems likely that ligand-receptor complexes flow through the MVBs and that as they do they are subjected to dissociating conditions such as transiently lowered pH. In a recent quantitative study Dunn *et al.*[19] showed that as more fluorescent tracer enters the endocytotic pathway, vacuolar endocytotic elements in the same size range as MVB become brighter rather than more numerous. It is possible, therefore, that several passages may be required before MVB processing (which in addition to dissociation might include conformational change, breakage of disulphide bonds and proteolytic cleavage)[3,20] is complete.

MVB movement and removal of membrane proteins

The view of the endosome system as consisting of an 'early' compartment processing rapidly recycling receptors and a 'late' compartment reached after longer intervals (<10 min) is compatible with the observations made in this study. It is now possible, however, to put this view in the context of a reticulum (through which ligand-receptor complexes moved at a rapid rate) and MVB/varicosities (into which some of these complexes become sequestered) in which they are then carried, more slowly, into the pericentriolar area. The rate at which the MVB are seen to move could depend upon a microtubular mechanism as suggested in previous studies[21,22].

Because EGF receptors are found initially on the perimeter membrane of the endosomal reticulum we conclude, as in our earlier studies[6,15], that the EGF-rich internal vesicles of the MVB arise by the invagination of receptor-rich domains on this boundary membrane. Movement of the MVB through the reticulum explains how such a large number of internal vesicles could be produced. The amount of membrane required to form these vesicles is far in excess of that available in the perimeter membrane of an isolated vacuole. In a moving varicosity the perimeter membrane will bring in a continuous supply of membrane. This process of 'microautophagy' suggests a system in which varicosities moving through the system gradually accumulate internal vesicles so that by the time they reach the pericentriolar area they will be loaded with internal membranes.

These studies, along with other recent reports of previously undetected cisternal compartments within exocytotic and endocytotic routes[9,23,24], suggest much greater continuity within intracellular pathways than did previous work which relied on chemically fixed preparations. They help to explain some apparent functional anomalies, such as the asynchrony of membrane trafficking which is frequently observed along these pathways, and they suggest that local sequestration within a cisternal compartment (rather than within separate vesicles) is likely to be a much more important feature of intracellular processing than has previously been realized. □

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LETTERS TO NATURE

DNH deoxyribonucleohelicates: self assembly of oligonucleosidic double-helical metal complexes

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NUCLEIC acids, because of their key biological role, are prime targets for the design of either analogues that may mimic some of their features or of complementary ligands that may selectively bind to and react with them for regulation or reaction. Whereas there has been much work on the latter topic since the elucidation of the double-helical structure of DNA[1,2], comparatively little has been done on structural and/or functional models, probably owing to the lack of self-organizing molecular systems. Here we present a class of artificial systems, the nucleohelicates, which are of interest from both points of view because they combine the double-helical structure of the double-stranded metal complexes, the helicates[2,3], with the selective interaction features of nucleic-acid bases. These functionalized species allow the study of structural effects on the formation of the double helix and on the binding to other entities, in particular to nucleic acids.

We have shown previously that oligobipyridine molecules containing a string of 2,2′-bipyridine (bipy) groups separated by a suitable spacer unit undergo spontaneous self-organization into oligonuclear metal complexes with a double-helical structure (Fig. 1). In these helicates two molecular strands are wrapped around each other and held together by Cu(I) ions that provide the driving force for organization by imposing a (distorted) tetrahedral coordination geometry at each site. Di- to pentahelicates have thus been obtained[2,3]. The trihelicate $[Cu_3(3\text{-}H)]^{3+}$, (4-H), schematically represented by structure $\mathscr{H}_3$, is formed by the binding of three Cu(I) ions by two tris-bipyridine molecules (3-H); it has been characterized by crystal structure determination[2]. These compounds provide a molecular framework for the spatial organization of substituent groups attached to the periphery of the double helix and projecting outwards. Exploitation of this possibility requires the introduction of functional groups on the bipyridine subunits that will allow the attachment of various molecular groups.

Two types of functionalized oligobipyridine compounds have been synthesized: fully substituted ones such as $\mathbf{3}\text{-}X_6$ and partially substituted ones, $\mathbf{3}\text{-}X_4$ and $\mathbf{5}\text{-}X_6$, where substituted and unsubstituted bipyridine units alternate along the strand (Fig. 2). They were obtained following a scheme similar to that used for the synthesis of the unsubstituted parent compounds **3**-H (ref. 2) and **5**-H (ref. 3) from the appropriate basic units (X, X′ = H, OH or Br; Y = CO_2t-Bu or $CH_2CH_2CO_2t$-Bu) derived from the diester **6** (X = X′ = H, Y = CO_2Me)[4] using reactions to be described elsewhere.

Compounds $\mathbf{3}\text{-}E_6$, $\mathbf{3}\text{-}(C_2E)_6$, $\mathbf{3}\text{-}(C_2E)_4$ and $\mathbf{5}\text{-}(C_2E)_6$ allow the introduction of a variety of groups (such as nucleosides, aminoacids, sugars, electroactive and photoactive units) on the oligobipyridine strands. Only one case, that of an aminodeoxynucleoside, 6′-amino-3′-deoxythymidine (H-Thy), used as its soluble silylated derivative (H-ThyS), is described here.

The corresponding derivatives $\mathbf{3}\text{-}T_6$, $\mathbf{3}\text{-}(C_2T)_6$, $\mathbf{3}\text{-}(C_2T)_4$ and $\mathbf{5}\text{-}(C_2T)_6$ represent artificial oligonucleosidic strands in which four or six nucleoside residues are attached to a backbone of bipyridine units. The analogous aminodeoxy-adenosine derivative $\mathbf{5}\text{-}(C_2A)_6$ has also been synthesized. Such molecules may, for instance, allow the study of base-pairing features in model arrays of nucleic acid components and the design of novel systems presenting recognition and self-assembling properties. They also represent alternatives to flexible oligonucleoside analogues[5]. Pendant nucleic-acid bases have been introduced on the backbone of organic polymers[6,7] and a number of systems containing two nucleic bases have been reported (see, for example, refs 8–14).

Complexation of Cu(I) by the unsubstituted oligobipyridine compounds **3**-H and **5**-H has been shown to give, respectively, the tri- and pentahelicates[2,3]. Similarly, the substituted derivatives described here gave the corresponding substituted helicates when the polyesters $\mathbf{3}\text{-}E_6$, $\mathbf{3}\text{-}(C_2E)_4$, $\mathbf{3}\text{-}(C_2E)_6$ and $\mathbf{5}\text{-}(C_2E)_6$ were treated with copper(II) trifluoro $CuTf_2$ (Tf, CF_3SO_3) (2 eq) and hydrazine ($N_2H_4 \cdot H_2O$) in CH_3CN or in $CH_3CN\text{-}CHCl_3$; these results will be described elsewhere. The 200-MHz ^1H-NMR and FAB^+ (fast-atom bombardment) mass spectra of the octaester and dodecaester helicates $\{Cu_3[\mathbf{3}\text{-}(C_2E)_4]_2\}Tf_3$, $\mathbf{4}\text{-}(C_2E)_8$ and $\{Cu_5[\mathbf{5}\text{-}(C_2E)_6]_2\}Tf_5$ are shown in Figs 3*a*, *c*, 4*a* and 5*a*; they agree with the structures, as do the elemental analyses.

A deep-red colour characteristic of Cu(I)-bipyridine complexes was also obtained on treatment of $\mathbf{3}\text{-}T_6$ with the same reagents in 1:1 aqueous acetonitrile. The NMR and mass spectral data did not confirm the formation of only the dodecathymidino-trihelicate, but indicated that a mixture of complexes was obtained. This may be due to the destabilizing effect of both the carbonyl groups conjugated with the pyridine rings and steric hindrance between the bulky substituent groups. Accordingly, we introduced a CH_2CH_2 segment into the side chains on alternating bipyridine units, as in $\mathbf{3}\text{-}(C_2T)_4$ and $\mathbf{5}\text{-}(C_2T)_6$, to reduce both adverse effects.

The following typical procedure was followed for preparing the complexes. 30.0 mg (1.35×10^{-5} mol) of $\mathbf{3}\text{-}(C_2T)_4$ were dissolved in 15 ml $CHCl_3$ at room temperature and a solution of 14.7 mg (4.06×10^{-5} mol, 3 eq) of copper(II) trifluoro $CuTf_2$ in 5 ml CH_3CN added. The blue-green solution of the Cu(II) complex thus obtained was stirred for 10 min. Then, addition of 30 µl (6.18×10^{-4} mol, 15 eq) of $N_2H_4 \cdot H_2O$ reduced Cu(II) to Cu(I) and gave the expected colour change to red. The reaction mixture was stirred for 15 min. After evaporation of the solvent, the residue was taken up in 20 ml $CHCl_3$ and filtered through cotton wool. By addition of 30 ml of ether, the product was isolated as a red precipitate (29.9 mg, 5.89×10^{-6} mol, 87%; m.p. 180–181 °C). Following the same procedure, treatment of 26.0 mg of **5**-$(C_2T)_6$ with 5 eq of $CuTf_2$ and 20 eq of $N_2H_4\cdot H_2O$ in CH_3CN-$CHCl_3$ gave 25.1 mg of a red-orange solid (m.p. 178 °C; 88% yield).

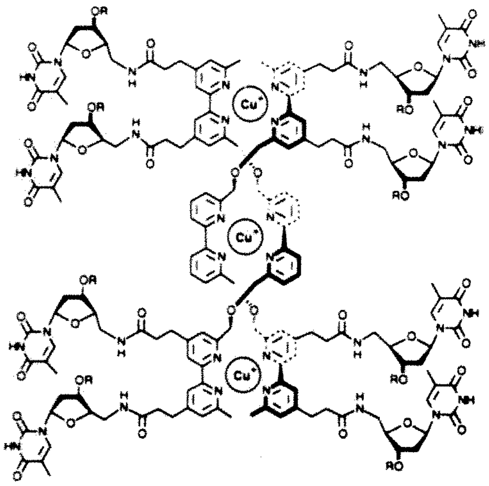
The elemental analyses (C, H, N, Cu) and ultraviolet absorption data of these solids were in agreement with the compositions $[\mathbf{3}\text{-}(C_2T)_4]_2[CuTf]_3$ and $[\mathbf{5}\text{-}(C_2T)_6]_2[CuTf]_5$.

The FAB$^+$ mass spectra, with peaks corresponding to $\{[\mathbf{3}\text{-}(C_2T)_4]_2Cu_3Tf_2\}^+$ (Fig. 3*b*) and $\{[\mathbf{5}\text{-}(C_2T)_6]_2Cu_5Tf_4\}^+$ (Fig. 3*d*), proved the presence of two ligands and, respectively, three and five copper(I) ions.

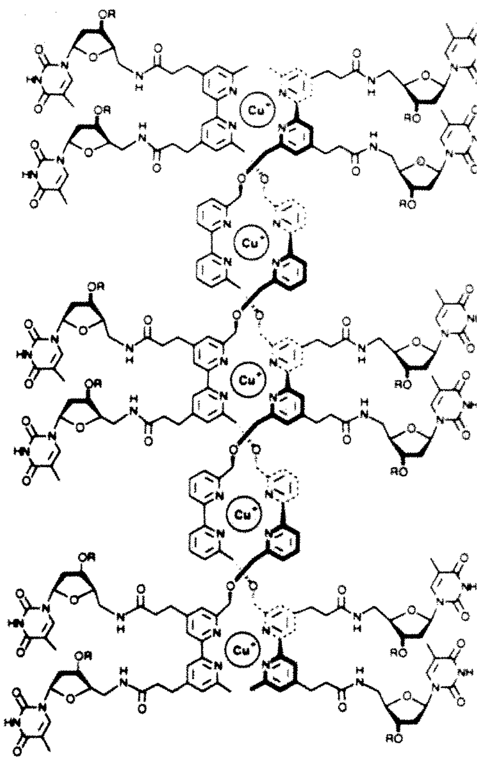
The ^{1}H-NMR spectra of the polynucleosidic trihelicate and pentahelicate were consistent with the assigned structures. High-field two-dimensional NMR techniques were used to analyse the spectra. In principle, complexation should lead to a mixture of the two diastereomers that differ in the handedness of the double helix. The presence of the chiral nucleosides might lead to a preferential induction of one helical sense, but this has not yet been proven. Figure 4*b* (left) shows the NMR and TOCSY (total correlation spectroscopy) spectra of $\{Cu_3[\mathbf{3}\text{-}(C_2T)_4]_2\}\ Tf_3$, compound **1**. Changes in the ligand chemical shifts upon copper complexation were consistent with those observed earlier[2] on formation of the unsubstituted trihelicate **4**-H from **3**-H. The first change is a marked upfield shift and splitting of the CH_2OCH_2 singlets into two multiplets (O) corresponding to tw AB systems in **4**-H. The cross-peak indicated in the TOCS' spectrum (O) results from the coupling in the two AB system: The second change is an upfield shift of the two bipyridin protons (3a, 3b) and of the bipyridine methyl group (Me); may be attributed to shielding by pyridine units of the othe strand. The interpretation was confirmed by comparison wit the ^{1}H-NMR spectrum of the octaester helicate **4**-$(C_2E)_8$ (Fi 4*a*, left), which shows similar chemical shifts and for which th same changes are observed on helicate formation.

In an analogous manner the ^{1}H-NMR spectrum (NMR an TOCSY) of $[\mathbf{5}\text{-}(C_2T)_6]_2[CuTf]_5$ (Fig. 4*b*, right) is consistent wit a dodecathymidino-pentahelicate $\{Cu_5[\mathbf{5}\text{-}(C_2T)_6]_2\}Tf_5$, **2**. Th most important spectral features are again the AB multiplet (O) (cross-peak O in the TOCSY spectrum). Comparison wit the spectrum of the corresponding dodecaester helicate $\{Cu_5[\mathbf{5}$ $(C_2E)_6]_2\}Tf_5$ (Fig. 4*a*, right) again confirms the structural an spectral assignments. Water-soluble derivatives of compound **1** and **2** (R = H, sulphate salts) have been obtained from th corresponding desilylated strands.

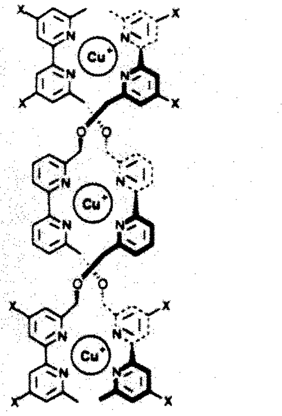
In view of its features this new class of substances may b termed deoxyribo-nucleohelicate, DNH. The DNH complexe: such as compounds **1** and **2**, are double-helical oligonucleoside formed spontaneously by self-assembling of two suitably designed ligand strands and copper(I) ions. In contrast to DNA the have positive charges located inside the strands whereas th information-bearing nucleic-acid bases are on the outer spin



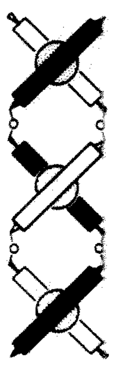
1 R = tBuMe$_2$Si



2 R = tBuMe$_2$Si

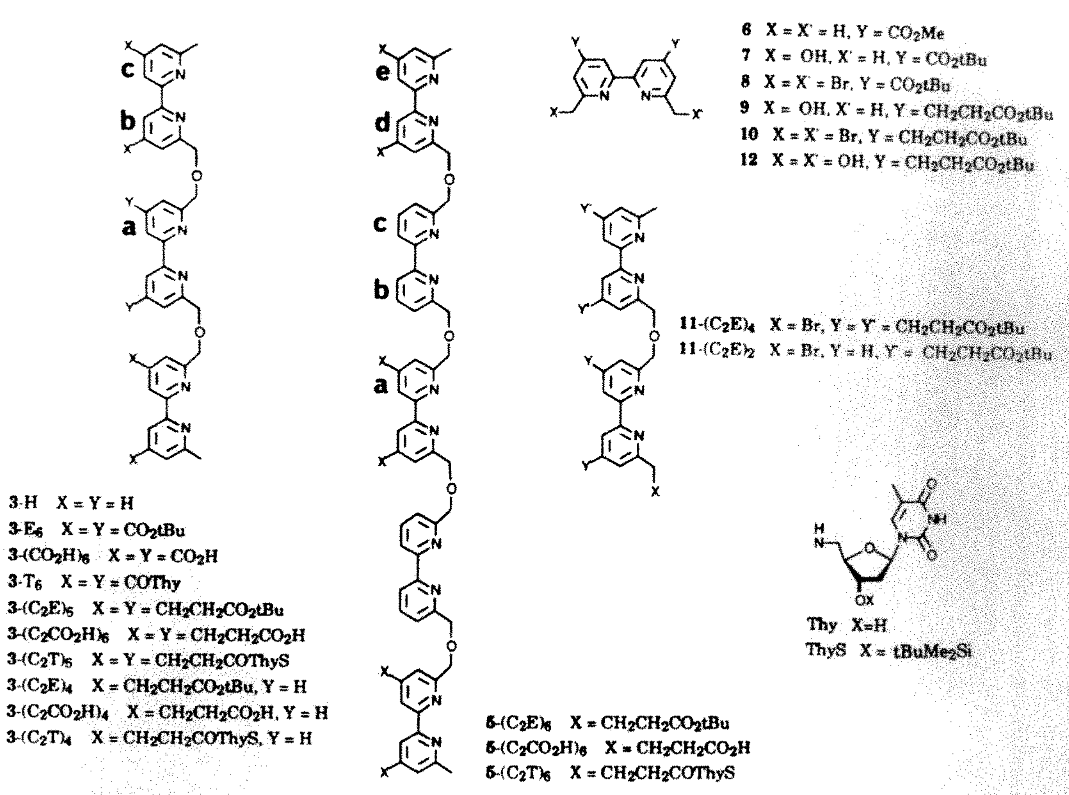


4-H X = H
4-$(C_2E)_8$ X = $CH_2CH_2CO_2tBu$



$\mathcal{H}_3$

FIG. 1 Structure of the deoxyribonucleohelicates **1** and **2** and of the trihelicates **4**-H and **4**-$(C_2E)_8$; the two bipyridine groups at each Cu(I) centre are almost perpendicular to one another, as schematically represented in $\mathcal{H}_3$ for **4**-H.



6 X = X' = H, Y = CO_2Me
7 X = OH, X' = H, Y = CO_2tBu
8 X = X' = Br, Y = CO_2tBu
9 X = OH, X' = H, Y = $CH_2CH_2CO_2tBu$
10 X = X' = Br, Y = $CH_2CH_2CO_2tBu$
12 X = X' = OH, Y = $CH_2CH_2CO_2tBu$

11-$(C_2E)_4$ X = Br, Y = Y' = $CH_2CH_2CO_2tBu$
11-$(C_2E)_2$ X = Br, Y = H, Y' = $CH_2CH_2CO_2tBu$

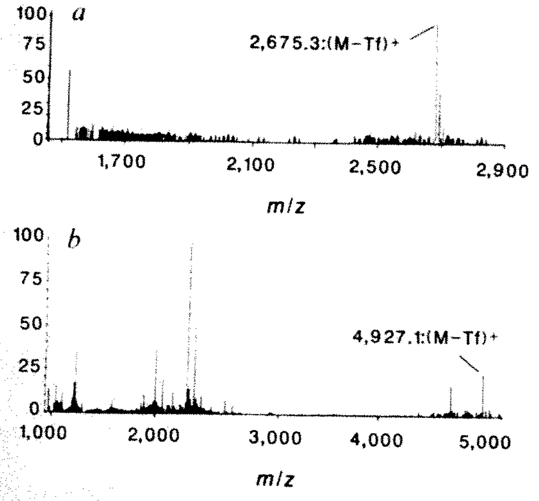
3-H X = Y = H
3-E_6 X = Y = CO_2tBu
3-$(CO_2H)_6$ X = Y = CO_2H
3-T_6 X = Y = COThy
3-$(C_2E)_5$ X = Y = $CH_2CH_2CO_2tBu$
3-$(C_2CO_2H)_6$ X = Y = $CH_2CH_2CO_2H$
3-$(C_2T)_5$ X = Y = $CH_2CH_2COThyS$
3-$(C_2E)_4$ X = $CH_2CH_2CO_2tBu$, Y = H
3-$(C_2CO_2H)_4$ X = $CH_2CH_2CO_2H$, Y = H
3-$(C_2T)_4$ X = $CH_2CH_2COThyS$, Y = H

5-$(C_2E)_6$ X = $CH_2CH_2CO_2tBu$
5-$(C_2CO_2H)_6$ X = $CH_2CH_2CO_2H$
5-$(C_2T)_6$ X = $CH_2CH_2COThyS$

Thy X=H
ThyS X = $tBuMe_2Si$

FIG. 2 Oligonucleosidic strands **3**-T_6, **3**-$(C_2T)_6$, **3**-$(C_2T)_4$ and **5**-$(C_2T)_6$ and synthetic intermediates.

METHOD. The key reaction was the condensation of compounds **7** and **8**. *t*-BuOK (2.4 eq) was added to a solution of compound **8** (2 eq) in *t*-BuOH and stirred at room temperature. After 1 h a solution of compound **7** (1 eq) in CH_2Cl_2 was added and the mixture was refluxed for 4 h. Together with the presence of the *t*-Bu groups in the ester functions, a bulky base and solvent were used to minimize side-reactions. The trimeric hexaester **3**-E_6 was isolated and purified by chromatography on silica (m.p. 135–136 °C, 50% yield). Condensation of 2 eq of the monoalcohol **9** with the dibromide **10** using *t*-BuOK (1:1 *t*-BuOH: tetrahydrofuran (THF), 20 °C, 24 h) gave elongated hexaester **3**-$(C_2E)_6$ (m.p. 76 °C, 31% yield) together with the bis-bipy monobromide **11**-$(C_2E)_4$ (oil, 23% yield), which were separated by column chromatography (alumina; eluent, 1:4 AcOEt: hexane). Condensation of 2 eq of the monoalcohol **9** with 6,6'-bis-bromomethyl bipy[20] using *t*-BuOK (1:1 *t*-BuOH:THF, 20 °C, 4 h) gave a mixture of the mono-substituted dimeric species **11**-$(C_2E)_2$ (m.p. 88 °C, 57% yield) and the alternating tris-bipy tetraester **3**-$(C_2E)_4$ (m.p. 48 °C, 30% yield), which were separated by column chromatography (alumina; eluent, 4:1 hexane:AcOEt). Condensation of 2 eq of the bis-bipy monobromide **11**-$(C_2E)_2$ with the dialcohol **12** (*t*-BuOK; 1:1 *t*-BuOH:CH_2Cl_2, room temperature 12 h) gave the alternating penta-bipy elongated hexaester **5**-$(C_2E)_6$ which was isolated and purified by chromatography (alumina; eluent, 2:1 hexane:AcOEt, m.p. 108 °C, 47% yield). 6'-Amino-3'-deoxy-thymidine (H-Thy) and its 3'-*t*-butyldimethyl silyl ether (H-ThyS) were prepared from thymidine following a reported reaction sequence[21]. The polyacids **3**-$(CO_2H)_6$, **3**-$(C_2CO_2H)_6$, **3**-$(C_2CO_2H)_4$ and **5**-$(C_2CO_2H)_6$ were obtained by acid-catalysed cleavage of the corresponding *t*-butyl esters (CF_3CO_2H, CH_2Cl_2, room temperature 12 h; >95% yield) and transformed into the corresponding acid chlorides ($SOCl_2$, reflux, 1–2h), which were used directly for condensation with either H-Thy or H-ThyS (2 eq per acid chloride function, 4 eq Et_3N, $CHCl_3$, 20 °C, 2 h) to give the corresponding oligonucleoside compounds **3**-T_6 (43% yield), **3**-$(C_2T)_6$ (143 °C, 48% yield), **3**-$(C_2T)_4$(167–168 °C, 70% yield) and **5**-$(C_2T)_6$(162–163 °C, 54% yield). The silylated compounds **3**-$(C_2T)_6$, **3**-$(C_2T)_4$ and **5**-$(C_2T)_6$, which were readily soluble in polar organic solvents (such as methanol, chloroform) were purified by column chromatography (silica, THF eluent). On the other hand **3**-T_6 had very low solubility in most common solvents except dimethyl sulphoxide (DMSO); its solubility could be increased by acylation of the free hydroxyl function to the hexa-acetyl, -butyryl, -methoxyacetyl and -decanoyl derivatives. All new compounds had spectral (NMR, FAB^+ mass) and elemental analysis data in agreement with their structure.



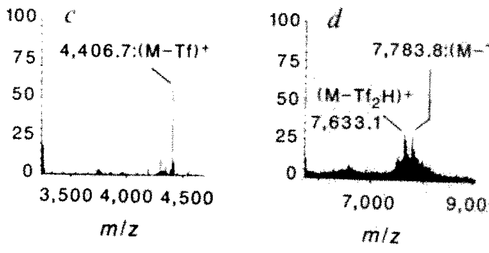


FIG. 3 Partial FAB^+ mass spectra of *a*, $Cu_3[$**3**-$(C_2E)_4]_2Tf_3$: $(M-Tf)^+$ found at 2,675.4 (calc. 2,675.3) (NBA, o-nitrobenzylalcohol); *b*, $Cu_3[$**3**-$(C_2T)_4]_2Tf_3$, **1**: $(M-Tf)^+$ found at 4,927.1 (calc. 4,926.5) (DMSO); *c*, $Cu_5[$**5**-$(C_2E)_6]_2Tf_5$: $(M-Tf)^+$found at 4,406.7 (calc. 4,406.3) (NBA); *d*, $Cu_5[$**5**-$(C_2T)_6]_2Tf_5$, **2**: $(M-Tf)^+$ found at 7,783.8 (calc. 7,782.8) (DMSO).

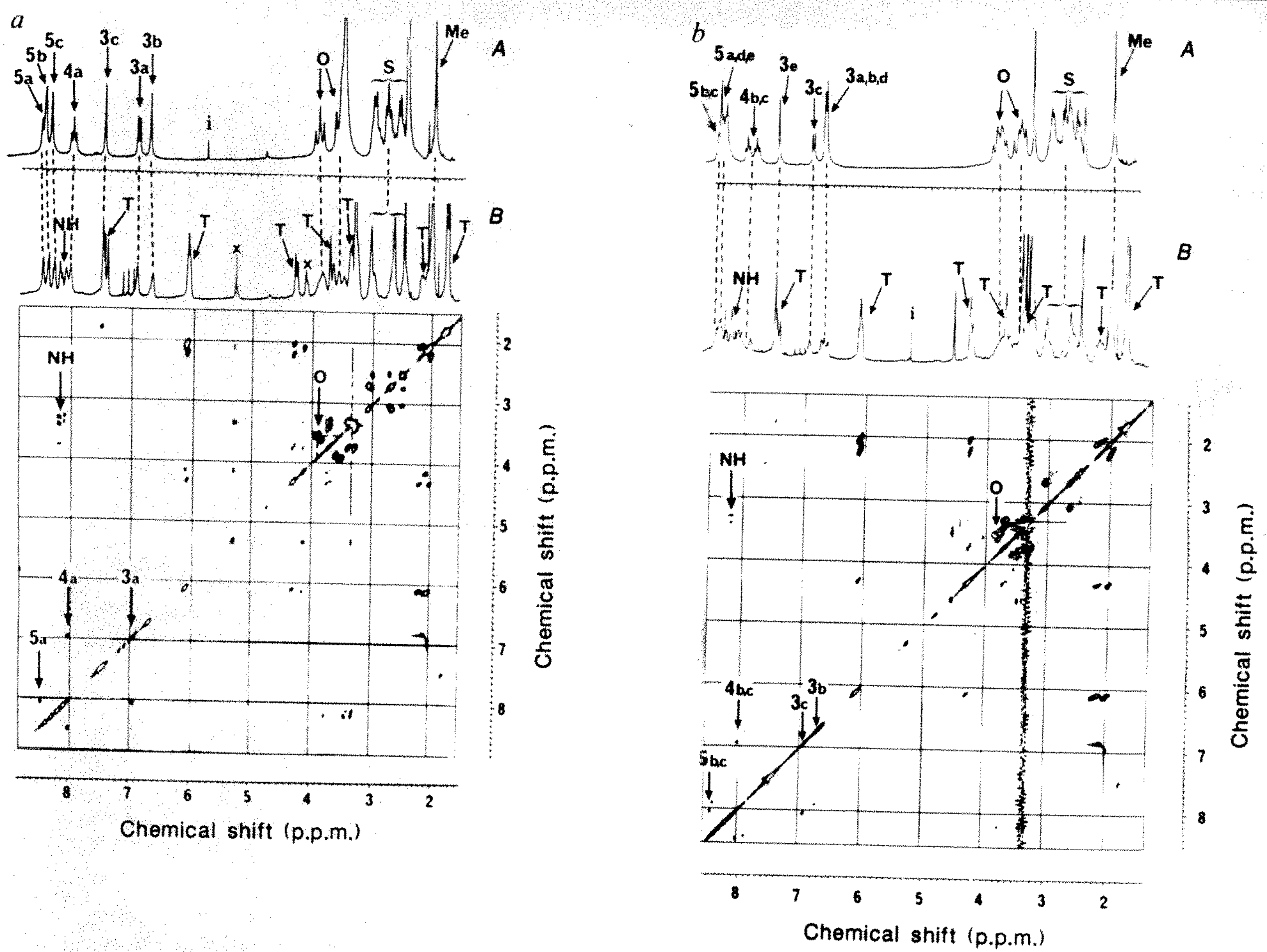


FIG. 4 ^{1}H-NMR spectra (200 MHz, 293 K) between 1.5 and 8.5 p.p.m. of the octaester trihelicate $(Cu_3(\mathbf{3}\text{-}C_2E_4)_2)Tf_3$ in d_6-DMSO (*aA*) and the dodecaester pentahelicate $(Cu_5\ (\mathbf{5}\text{-}(C_2E)_6)_2)Tf_5$ in d_6-DMSO (*bA*) showing the aromatic region (3, 4, 5), the methylene oxy bridge (O), the two-carbon spacer(S) and the bipyridine methyl group (Me) signals. ^{1}H-NMR (600 MHz, 293 K) and TOCSY spectra of the octathymidine trihelicate **1** in d_6-DMSO (*aB*) and the dodecathymidine pentahelicate **2** in d_6-DMSO (*bB*). Helicate signals having the same chemical shift as in *aA* and *bA* are indicated by dashed lines, thymidine signals by T, and propionamide NH by NH. Cross-peaks are marked by arrows for protons 3, 4, 5 on the bipyridine ring systems a–e, the propionamide NH and the methylene oxy bridge AB systems. x, thymidine monomer impurity; triplet at 7.1 p.p.m., NH_4^+; solvent peaks at 2.5 (DMSO) and 3.5 or 3.3 p.p.m. (H_2O); i, solvent impurity; a–e refer to the different pyridine rings (see Fig. 2).

of the double helix, pointing away from its axis; this structure recalls the inside-out model once proposed for DNA[15]. They should be able to interact with other species both electrostatically and by hydrogen bonding through the thymidines (in a metallonucleate exoreceptor[16] fashion). One possible target is the negatively charged double-stranded DNA; binding in its major groove would lead to a composite natural–artificial quadruple-helix entity which might present thymine·adenine·thymine interactions (of the triple helix type[17-19]) with adenine-thymine regions in the DNA. Such possibilities are being explored. □

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Prediction of crystal structures from crystal chemistry rules by simulated annealing

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THE prediction of the structure of inorganic crystalline solids from the knowledge of their chemical composition is still a largely unresolved problem[1–3]. The usual approach to this problem is to minimize, for a selection of candidate models, the potential energy of the system with respect to the structural parameters of these models: the solution is the arrangement that comes out lowest in energy. Methods using this procedure may differ in the origin (*ab initio* or empirical) of the interatomic potentials used, but they usually restrict themselves to optimizing a structural arrangement within the constraints of given symmetry and bond topology. As a result, they do not truly address the problem of predicting the unknown structure of a real compound. The method we describe here is an attempt at solving the following problem: given the chemical composition of a crystalline compound and the values of its unit-cell parameters, find its structure (topology and bond distances) by optimizing the arrangement of ions, atoms or molecules in accordance with a set of prescribed rules. The procedure uses simple, empirical crystal chemistry arguments (Pauling's principles for ionic compounds[4]) and a powerful stochastic search procedure, known as simulated annealing[5] to identify the best atomic model or models. We discuss the potential of the method for structure determination and refinement, using results obtained for several known inorganic structures, and by the determination of a previously unknown structure. Although the approach is limited to the case of inorganic compounds, it is nevertheless very general, and would apply to any crystalline structure provided that the principles governing the architecture of the solid can be properly described.

The structures of almost all known inorganic compounds are consistent with the simple crystal chemistry principles put forward long ago[4]. Although these rules have been used extensively to assess the exactness and/or accuracy of newly determined structures[6], their use to predict atomic-scale structural arrangements has been very limited. Recently Brown[7] has developed a two-step method: (1) by trial and error, a three-dimensional model is built of the strongly bonded complexes, chosen using the nature of the elements; (2) the geometry is refined using a distance-least-squares (DLS) refinement[8]. The first step cannot easily be automated and must for the moment be performed by an expert operator.

The method described here, which focuses on ionic compounds, uses a different approach: the reasonableness of an ionic arrangement in an experimentally known unit cell is described by a 'cost' function which quantifies its departure from a set of building rules. To avoid getting trapped in local minima, this cost function is globally minimized by simulated annealing[5]: starting from a randomly generated arrangement and very slack constraints this algorithm progressively enforces the rules until a (hopefully) minimal-cost configuration is obtained. The only intervention required is the input of information (chemical formula, cell dimensions and valence-bond parameters) and control of the annealing program, which defines how rapidly the rules are enforced. This method deliberately ignores symmetry constraints (except the shape and size of the simulation box) and, at variance with most methods for structure modelling, treats all ions in the unit cell as independent species. A related technique of crystal structure determination, based also on simulated annealing but applied to zeolites and using a different cost function, has been recently described[9].

The details of simulated annealing have been described in a number of reviews (see, for example, ref. 10). Briefly, four items are required to apply the algorithm: (1) a description of the system, that is, a set of parameters describing the size and shape of the unit cell, and the bond-valence characteristics of the ionic species. The initial coordinates are normally produced by a random number generator; (2) a cost (or energy) function—a scalar function that reduces the objective of the optimization to a single number; (3) a procedure to explore the configuration space of the system—this is achieved by a Monte Carlo technique using Metropolis importance sampling[11]. Here, temperature is the control parameter; (4) a cooling (or annealing) schedule—the simulation starts at high temperature where almost all moves are accepted (liquid state) and several moves are attempted until

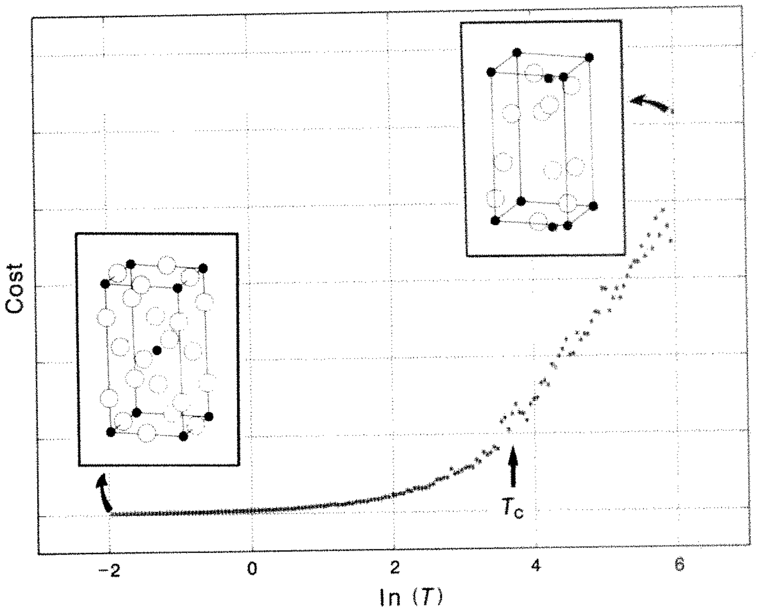

FIG. 1 Cost function E as a function of the logarithm of temperature during the annealing of NbF_4 structure. Bond-valence parameters: $r_0 = 1.96$ Å, $\rho = 0.27$ Å. Simulated-annealing parameters: initial 'temperature' $T_0 = 400$ (arbitrary units); cooling rate $\alpha = 0.95$; maximum move allowed $\delta_0 =$ 0.3 Å (initial), $\delta_f = 0.002$ Å (final); δ was regularly updated to keep acceptance rate above 30%. To improve the statistics of the maps of the radial distribution functions the simulation was performed with 5,000 Monte Carlo cycles per temperature but convergence to the correct structure can be achieved with only 400 cycles per temperature. The weighting factor λ between the two terms of the cost function, calculated from the high-temperature part of the simulation, was ~0.85. Inset: structural arrangement of NbF_4 at the beginning of the simulation (right) and as obtained by simulated annealing (left).

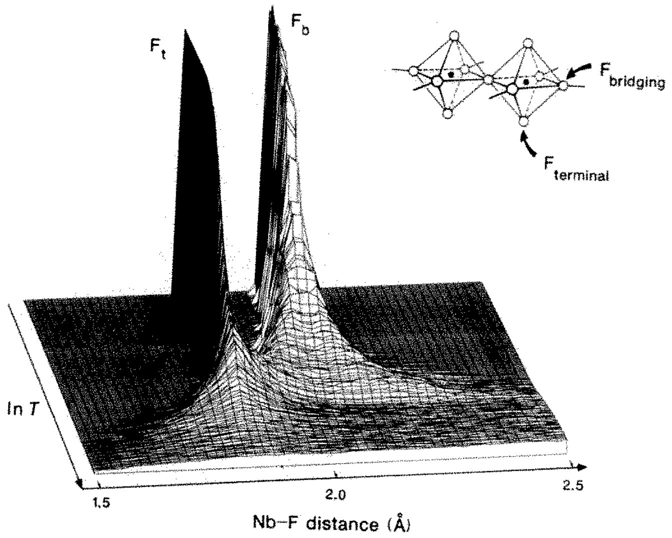

FIG. 2 Evolution of Nb–F radial distribution function in NbF$_4$ during annealing from 'molten' to crystalline state. The strong (short) Nb–F$_{terminal}$ bond freezes at higher temperature than does the weaker (long) Nb–F$_{bridging}$ bond.

the system reaches thermal equilibrium; the temperature is then reduced and the process is repeated until the system becomes frozen. We used a simple annealing program[5] defined by the logarithmic law $T_{t+1} = \alpha T_t$ with $0.8 < \alpha < 1$.

We have used the electrostatic valence principle[4] in its bond valence/bond length formulation[6] to generate the cost function. The first term is defined as the overall departure from this rule, that is, the overall bond-valence unbalance of the configuration:

$$E_{\text{valence}} = \sum_{i}^{\text{cell}} \left| q_i - \sum_{j=1}^{\text{CN}_i} S_{ij} \right|$$

where q_i is the valence (formal charge) of ion i, CN_i its coordination number and s_{ij} the valence (strength) of bond ij; s_{ij} is linked to interatomic separation r_{ij} by an usual empirical expression of the form $s_{ij} = \exp[(r_0 - r_{ij})/B]$ where r_0 and B are constants for a particular ion pair[6]. Coordination numbers are defined by applying a spherical cutoff to exclude from the second sum all neighbours j giving a contribution s_{ij} smaller than an arbitrary threshold (typically $0.02|q_i|$). In contrast to most energy functions used in structure modelling, the above expression does not refer to single pair interactions but involves the complete coordination sphere of every atom in the structure. This rule of local equilibrium controls the coordination of cations by anions and vice versa but does not restrict cation–cation or anion–anion packing. An elementary but not general method to constrain these two packings is through an electrostatic-like interaction of the form

$$E_{\text{electr}} = \frac{1}{2} \sum_{i,i'} \frac{q_i q_{i'}}{r_{ii'}}$$

where the indices i, i' cover all cation–cation and anion–anion pairs in the unit cell (using the minimum-image convention for truncation of the potential). Introducing a coefficient λ to express the relative cost of the two contributions, we obtain the total cost function $E = (1-\lambda)E_{\text{valence}} + \lambda E_{\text{electr}}$. The choice of λ is rather critical for many structural types; we found that it can be conveniently estimated during the early stages of the simulation from the variance V of the two terms of the cost function: $\lambda = V_{\text{valence}}/[V_{\text{valence}} + V_{\text{electr}}]$ where $V_k = \langle E_k^2 \rangle - \langle E_k \rangle^2$.

The computing time depends both on the size of the simulated sample and on the annealing process. Starting from an arbitrary model, a typical run for a 10-atom structure (see NbF$_4$ below) consumes ~20 min of CPU (central processing unit) time on a VAX-8700.

Figures 1 and 2 show the performance of the method in th case of a simple compound, NbF$_4$. The crystal structure consist of a tetragonal stacking of layers of corner-sharing octahedra[1] (see inset in Fig. 1). Although of modest complexity (10 atom per unit cell) it exhibits two topologically different fluorin atoms and illustrates the essential features of the method. Th progress of the simulation can be followed by the energy (Fig 1) or by the radial distribution functions (Figs 2, 3). At the beginning of the simulation (high temperature), almost al configurations are allowed: the average cost function is hig and the partial radial distribution functions are structureless On cooling, short-range ordering progressively builds up an broad peaks appear which rapidly become narrower as the system freezes into a configuration that corresponds closely t the experimentally observed structure: the 12 independent Nb–F distances of the simulated structure differ by ≤0.04 Å from the two experimental values. An increase in the variance of the cost function signals the onset of the 'crystallization' (Fig. 4). It is interesting to note that when non-optimized values of the weighting factor λ are used or when the cooling is not properly performed, a molecular structure consisting of a packing of NbF$_4$ tetrahedra (similar to the structure of SnI$_4$) may be obtained.

The algorithm has been tested successfully on a variety of simple problems including three-dimensional structures (rock salt, rutile, fluorite, perovskite, BaSiF$_6$) and layer structures (KAlF$_4$, K$_2$NiF$_4$). The structure of the first dirutile compound LiCoF$_4$ was actually solved by this method[13] and later confirmed by neutron powder diffraction[14]. The magnetic structure of this compound was also predicted from postulated exchange integrals, using a method[15] similar to this one.

At present the method is not efficient enough for use in most practical problems of *ab initio* structure determination. The optimization of the cooling schedule for continuous problems is still largely unsolved, leading to prohibitive amounts of computing time for structures with a large number of atoms or with a complex cost landscape (large number of local minima). But most of the present limitations of the method result from the

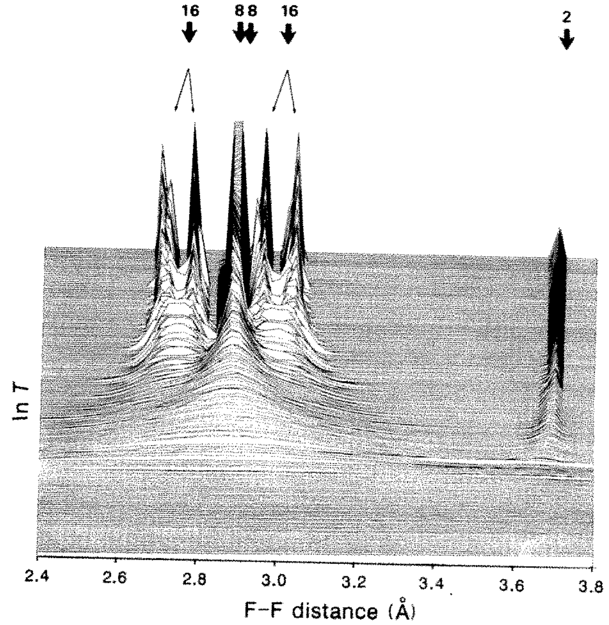

FIG. 3 Temperature-dependence of the F–F radial distribution function in NbF$_4$. The arrows and numbers at the top indicate the experimentally observed distances and their multiplicities[12]; the topology obtained is correct but the limited accuracy of the bond valence parameters as well as small fluctuations in the bridging fluorine positions at low temperature split two of the equivalent F–F distances in two subgroups.

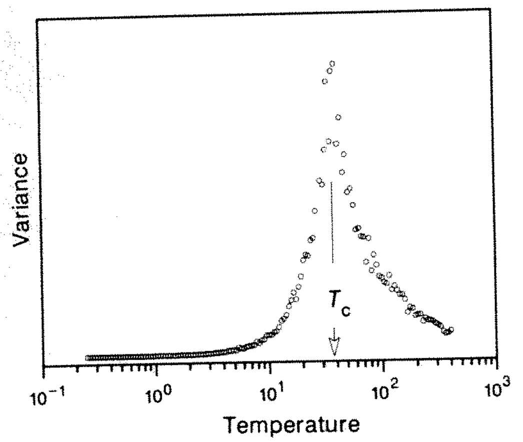

FIG. 4 Variance of the cost function of NbF_4 during the annealing process. The arrow indicates the 'crystallization' temperature.

simple cost function used. First, the 'naive' second term of the cost function we used performs well for ionic compounds that can be described as a close packing of spherical atoms but fails when applied to cations that form strongly directional bonds. For the same reason the cost function is not suited to structures exhibiting face-sharing polyhedra with highly charged cations (such as α-Al_2O_3). Second, within the accuracy of the valence-bond rule (and parameters), different topological configurations are sometimes obtained for some compounds. This emphasizes the weakness of current empirical expressions used to correlate bond valence and bond lengths: their accuracy is adequate to check the validity of an experimentally determined atomic arrangement[6] but we found that it is not always sufficient for reliable *ab initio* structure prediction. Other expressions such as that recently introduced by Hoppe *et al.*[16] to calculate charge distributions in crystals may provide interesting alternatives. Another fruitful approach would be to explore the apparent similarity[17] of the bond valence network to electric circuits and introduce the analogue of Kirchhoff's voltage law in the cost function. This ring sum rule, advocated by O'Keeffe[18], provides further constraints which could complement or even replace the electrostatic term now used in the cost function.

Despite the current limitations, the results mentioned above demonstrate that the simulated annealing approach to structure prediction is a promising method for exploring the possible arrangements that are compatible with a set of prescribed building rules within the limits of a given unit cell. As such, the method is of general applicability: the challenge is to conjecture the empirical or semi-empirical rules that govern the architecture of the solids. ☐

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Phase transition in polymer gels induced by visible light

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PHASE transitions and critical phenomena in polymer gels have attracted much attention because of their scientific interest and technological significance[1–3]. Phase transitions accompanied by a reversible, discontinuous volume change as large as several hundred times, in response to infinitesimal changes in environmental conditions, have been observed universally in gels made of synthetic and natural polymers[1–9]. Phase transitions have been induced in gels by varying temperature, solvent composition, pH, ionic composition and a small electric field[10]. Recently, gels sensitive to ultraviolet light were also reported[11]. The ultraviolet light initiates an ionization reaction in the gel, creating internal osmotic pressure which induces swelling. In the absence of this light, the equilibrium tends towards the neutral polymer system and the gel collapses. This transition process is slow, as it depends on the photochemical ionization and subsequent recombination of ions, and it is technologically desirable that the transition be induced by faster mechanisms. Also, visible light is less harmful and more abundant than ultraviolet in sunlight. Hence we now report the phase transition of gels induced by visible light, where the transition mechanism is due only to the direct heating of the network polymers by light, which is an extremely fast process. Such systems might be used as photoresponsive artificial muscles, switches and memory devices.

Gels containing *N*-isopropylacrylamide (main constituent) and the light-sensitive chromophore, trisodium salt of copper chlorophyllin (Fig. 1), were formed by free-radical copolymerization as follows: 7.8 g of recrystallized *N*-isopropylacrylamide (Kodak), 0.67 g of *N,N'*-methylenebisacrylamide (cross-linker, Bio-Rad), 0.72 g of chlorophyllin (Aldrich) and 240 μl of tetramethylethylenediamine (accelerator, Bio-Rad) were dissolved in 100 ml of degassed water at 0 °C, to which 0.2 g of ammonium persulphate (Mallinckrodt) was added to initiate the polymerization. Capillaries of ~100-μm inner diameter were

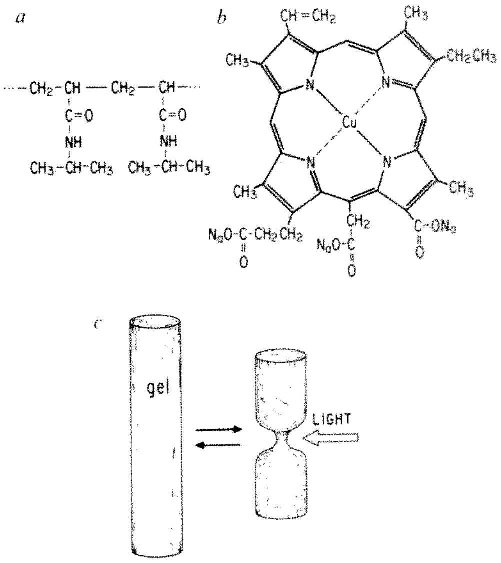

FIG. 1 Chemical structures of *N*-isopropylacrylamide (*a*) and trisodium salt of copper chlorophyllin (*b*). Chlorophyllin has a double bond that can be covalently connected to the polymer network. *c*, The collapse of the gel under illumination.

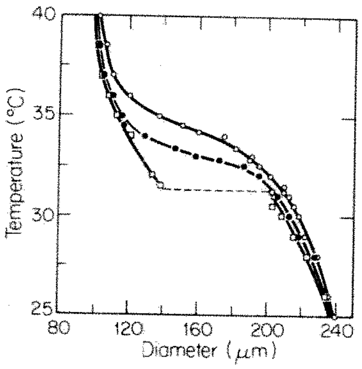

FIG. 2 Diameter of *N*-isopropylacrylamide/chlorophyllin copolymer gel as a function of temperature. Open circles, I=0 mW; solid circles, I=60 mW; squares, I=120 mW 488-nm radiation.

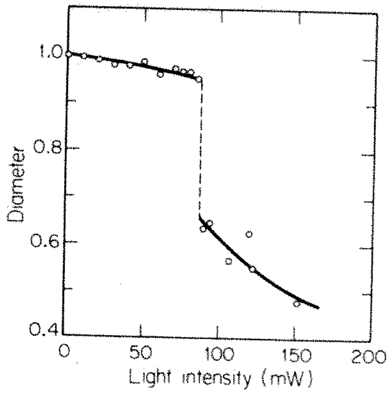

FIG. 3 Diameter of *N*-isopropylacrylamide/chlorophyllin copolymer gel as function of intensity at 31.5 °C. The data were taken on the gels with th same composition but with an initial diameter different from the one use in Fig. 2.

inserted into the solution. After gelation, the cylindrical samples were removed from the capillaries and dialysed extensively with distilled deionized water. A gel sample was then immersed in water (pH 5.8) or a NaOH solution (pH 11.9) and sealed in a rectangular glass microcapillary, the temperature of which was regulated to within ±0.1 °C. Argon-ion laser radiation at 488-nm was used, and the light intensity at the gel was varied from 0 to 150 mW using a polarizer. The incident beam, gaussian diameter ~7 mm, was focused to 20 μm (focal depth 0.8 μm) at the sample using a lens of 19-cm focal length. The size and shape of the gel were then monitored and analysed using an image processor (Model C1966, Hamamatsu Photonics).

Figure 2 shows the equilibrium volume of the gel at pH 11.9 as a function of temperature for three values of light intensity. In the absence of illumination, the gel underwent a sharp but continuous volume change at ~35 °C. At a light intensity of 60 mW, the volume change became more pronounced and the 'bulk' equilibrium transition temperature was lowered to 33 °C. At an intensity of 120 mW the gel showed a discontinuous volume transition at 31.5 °C. The data in Fig. 2 show that the light-sensitive polymer gel is swollen in the absence of irradiation but collapses when illuminated with light of visible wavelength. Light shrank the gel over the entire temperature range, but the largest effect was seen in the transition region. These features were observed at both pHs. The results indicate that at a appropriate 'bulk' equilibrium temperature the gels shoul undergo a discontinuous transition when the light intensity i varied, as we observed when we fixed the temperature at 31.5 ° and varied the intensity from 0 to 150 mW (Fig. 3). The ligh intensity at the transition varies from gel to gel, as seen in th difference between Figs 2 and 3. The differences in the ratio of gel and beam diameters, or in the bleaching conditions ma be responsible for the variation.

Two interesting features of the light-induced phase transitio are that irradiation causes the originally continuous transitio to become discontinuous and causes the transition temperatur to be lowered. These can be explained using the Flory–Huggin equation of state. The swelling curve given by the condition o zero osmotic pressure for the gel is[8,12]

$$\frac{1}{T}=\frac{\Delta S}{\Delta H}+\frac{k}{\Delta H}\left[\frac{v_1\nu_c}{N\phi^2}\left\{(2f+1)\left(\frac{\phi}{\phi_0}\right)-2\left(\frac{\phi}{\phi_0}\right)^{1/3}\right\}-\frac{2}{\phi}-\frac{2\ln(1-\phi)}{\phi^2}\right]\qquad(1)$$

where T is the absolute temperature, ΔS and ΔH are the entrop and enthalpy of polymer–polymer contact, k is the Boltzmann

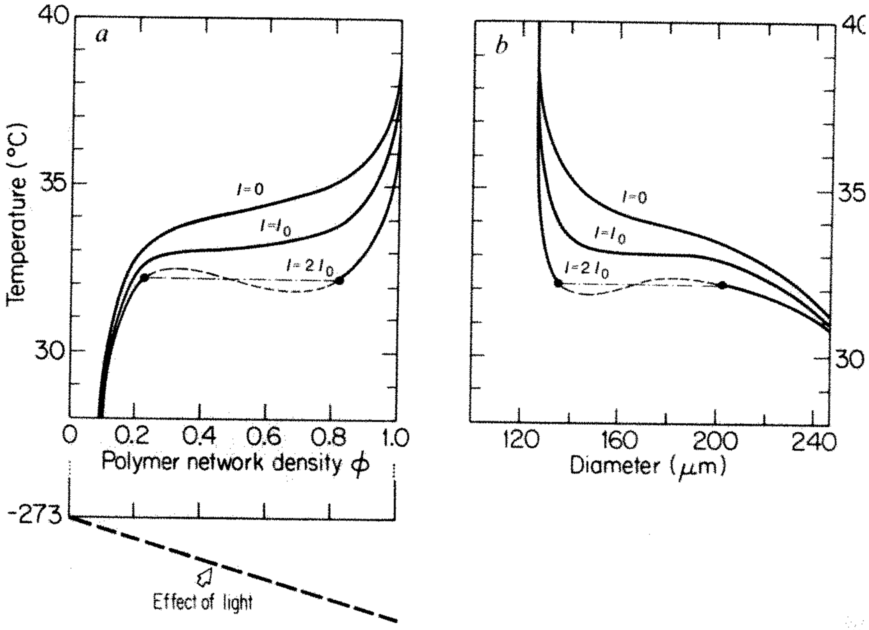

FIG. 4 *a*, Theoretical swelling curves of a gel for various light intensities, constructed using the equations in the text. The effect of illumination is to add a linear distortion of negative slope in proportion to the polymer density. *b*, Theoretical swelling curves as in *a* except that the horizontal axis is reduced diameter $d=d_0(\phi_0/\phi)^{1/3}$.

constant, N is Avogadro's number, v_1 is the molar volume of water, ν_e is the total number of the effective polymer chains in the gel, ϕ is the polymer network density, ϕ_0 is the density when polymers are in the random-walk configuration, and f is the number of counter-ions per chain. For N-isopropylacrylamide gels the interaction parameters ΔS and ΔH are both negative.

For simplicity we rewrite equation (1) as

$$T = T_{\mathrm{gel}}(\phi) \tag{2}$$

This function increases monotonically with network density ϕ for small ionization f of carboxyl groups of chlorophyllin. When the gel is irradiated, the chromophore absorbs the light, which is then dissipated locally as heat by radiationless transitions, increasing the 'local' temperature of the polymer. The temperature increment should be proportional to the light intensity and the chromophore concentration, and therefore to the polymer network density ϕ. Thus

$$T = T_0 + \alpha I \phi \tag{3}$$

where T_0 is the ambient temperature, I is the light intensity, and α is a constant, ~0.6 °C at $I = 100$ mW (Y. Li, personal communication). Combining equations (2) and (3)

$$T_0 = T_{\mathrm{gel}}(\phi) - \alpha I \phi \tag{4}$$

As illustrated in Fig. 4, the effect of the second term in equation (4) is to bring the gel state into the unstable region in the $T_0 - \phi$ space, inducing a discontinuous transition and to lower the transition temperature. Theoretical swelling curves and the contribution by light $(+\alpha I \phi)$ to them are shown in Fig. 4. Because thermal diffusion is much faster than collective diffusion of the gel network, the swelling and shrinking process of the gel in response to light is governed only by the motion of the polymer network. For 1-μm-diameter gels the response time[13,14] is expected to be ~5 ms. ☐

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Temperature and rainfall estimates for the past 40,000 years in equatorial Africa

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THERE has been considerable debate about the magnitude of the decrease in temperature[1,2] and the change in precipitation[3] in the African tropics during the last glacial period. With the advent of fossil pollen studies in equatorial regions, it is now generally agreed that the temperature did decrease at this time in tropical regions[4], but the magnitude of the temperature fluctuations and discrepancies between the continental[5] and marine[6] temperature records have yet to be resolved[7]. Here we present new quantitative estimates of temperature and precipitation using a multivariate analysis[8] of pollen time-series data from peat deposits in Burundi for the past 40,000 years[9]. For the last glacial period, our estimate of a temperature decrease of 4 ± 2 °C is less than those (ranging from 5 to 8 °C) derived from snow-line and tree-line records[5,7]. Model simulations[7] indicate that the snow-line and tree-line estimates (from high-elevation sites at ~4,000 m above sea level) are incompatible with the marine temperature record. Our lower estimate from a site of intermediate elevation may help resolve the differences between these records. We also estimate that the mean annual rainfall decreased by 30% during the last glacial period, in agreement with the rainfall history inferred from lake level fluctuations[10].

Pollen data contains climate information which can be extracted using a variety of statistical techniques[11,12]. Although the response of vegetation to climate needs further investigation[13], pollen data have already provided successful quantitative estimates of climate parameters for temperate regions in North America[12], India[14] and Europe[15,16]. Here we focus on a fossil sequence recently obtained in the mountains of the Zaire/Nile divide in Burundi[9], which provides new data for the region south of the Equator[17]. Our statistical procedure has been described in detail elsewhere[18].

The modern data set that we used is from East Central Africa between latitude 4° S and 12° N and longitude 28° and 42° E[19]. It contains pollen samples from 356 sites that cover most plant communities from desert to subalpine grasslands and includes all types of forests[20]. Mean annual rainfalls range from 200 to 2,000 mm (ref. 21). The decreasing temperature gradient is registered by the change with altitude of the vegetation on high mountains[22] (Fig. 1).

For meteorological data, we compiled means of the modern measured values (normals) for about thirty years[23]. To adjust data from the meteorological stations to the modern pollen sampling sites, an interpolation of temperature and precipitation values was carried out when necessary, according to the rules and procedure previously described[8]. Although the seasonal pattern linked to the movements of the Inter Tropical Convergence Zone is important in the tropics[24], we have focused on reconstructing the mean annual climate and have used the mean annual temperature and rainfall. The reconstruction of a palaeoclimate from pollen data relies on the assumption that climate is the main factor forcing changes in the vegetation and therefore pollen frequencies[13]. This is a subject of some debate, but we consider it to be valid for the 100–300 yr resolution of the fossil sequence.

Multiple regression shows that the modern-pollen database contains a significant climate signal: the correlation of estimated values extracted from pollen data to observed meteorological data was 0.90 for temperature and 0.80 for rainfall[19]. Although the regression method has been successfully applied to pollen data from temperate regions[11,12,16], it has gradually been replaced because it does not separate the different components of the climate signal. Best-analogues methods, such as surface response[25] and palaeobioclimate analogues[15] have now been shown to yield plausible palaeoclimate estimates.

The analogue approach consists of looking for modern pollen data similar to the fossil ones and of equating the appropriate modern climate to the palaeoclimate. The statistical distance is a measure of the difference between the modern and the fossil-pollen assemblages[17]. In our palaeobioclimate approach, this

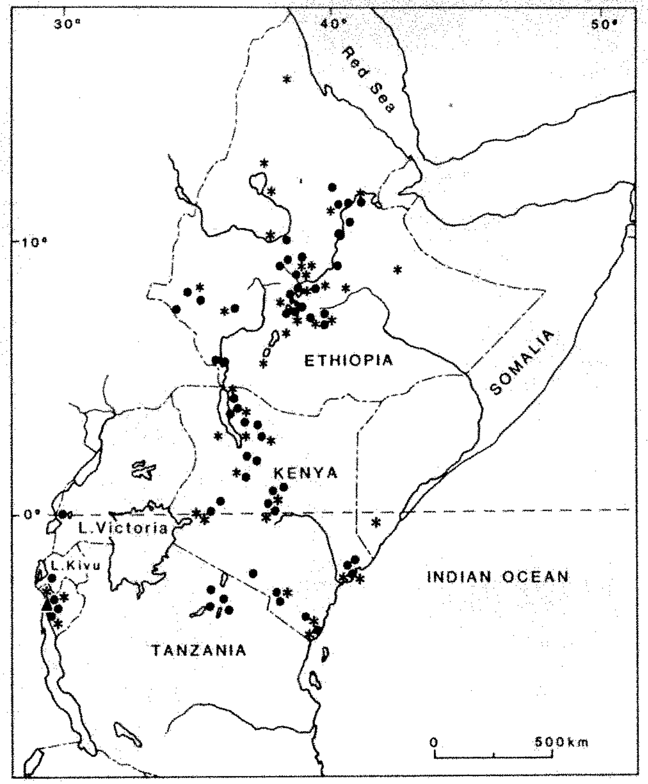

FIG. 1 Location map of the modern pollen samples used for reconstructio of the palaeoclimate*, Meteorological station sites; ●, Modern pollen; ▲ core site.

has two factors. First, the pollen percentages of both the modern and the fossil data are transformed into logarithms to smooth the variations of the over-represented pollen taxa. Second, a statistical weight is attached to each pollen taxa. If we had used modern pollen data to calculate these weights, they could have been biased owing to human disturbance of the relation between pollen frequencies and climate, even if much-disturbed vegetation had been avoided by selective sampling. Unlike other studies, we calculate these weights using a time-series analysis of the fossil sequence. The weights are given by the first eigenvector of the cross-correlation matrix[26] between the different pollen taxa of the fossil sequence. As in the case of the first principal component of the standard correlations between taxa, this first eigenvector reflects the major variations of the fossil pollen data. Moreover, we use cross-correlations at lag 1 (that is, correlations between successive depth levels) to emphasize those taxa with a coherent temporal behaviour. The first eigenvector is then presumed to be a good monitor of the evolution of climate.

In the time-series analysis of this fossil sequence, the first eigenvector accounts for >60% of the sum of the squared

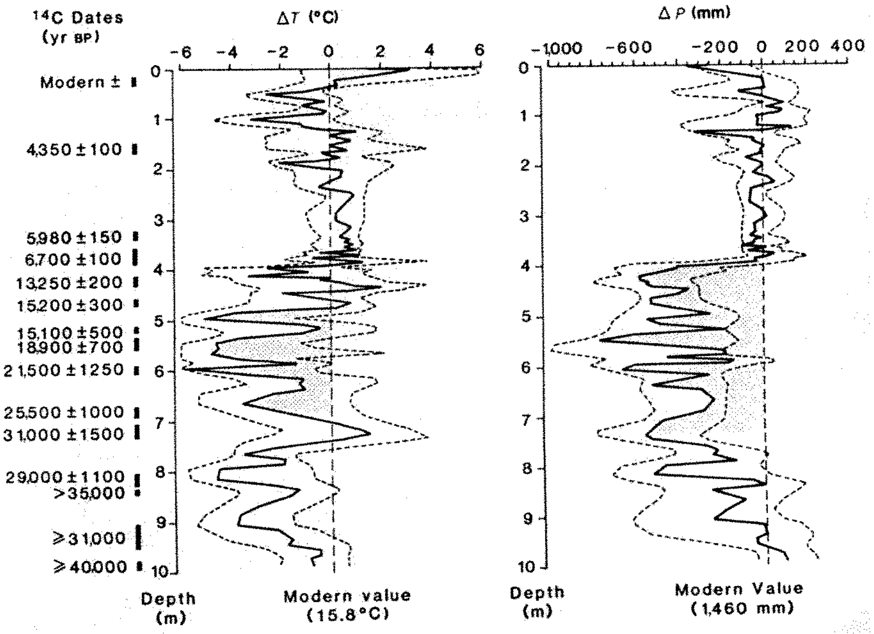

FIG. 2 Reconstructed mean annual temperature and rainfall at Kashiru, 3°28′ S, 29°34′ E, Burundi, expressed as deviation from the modern value. The dashed lines represent the mean of lower and upper standard deviations.

autocorrelations of the pollen percentages of 70 selected pollen taxa. Table 1 lists those that dominate the first eigenvector and the statistical distance between the modern and the fossil pollen spectra.

The reconstructed temperature and rainfall estimates are obtained by averaging the values of the best 20 modern analogues, weighted by the inverse of the squared distance. These calculations were done for each of the pollen assemblages at individual stratigraphic levels of the fossil sequence. The modern analogues with a temperature less than this average provide the lower standard error and the analogues with a greater temperature provide the upper one. This results in an asymmetrical confidence interval around the mean reconstructed climate parameters. The mean errors are found to be 2.2 °C for temperature and 200 mm for precipitation, both with a probability of 0.70. The curves in Fig. 2 have been smoothed by a three-point digital filter to avoid fluctuations limited to one or two samples.

The quality of the reconstructions was tested by estimating the climate parameters at the modern pollen samples sites using the same modern analogues and the weights of pollen taxa previously calculated from the fossil sequence. The correlations between the estimated and measured meteorological values, have coefficients of 0.94 for temperature and 0.85 for precipitation, indicating that the reconstructions are soundly based. The statistical significance of this test is high, especially in light of the fact that the weights were calculated from the fossil data, not from the modern pollen spectra.

Figure 2 gives the mean annual temperature and rainfall curves reconstructed from the 40-kyr pollen sequence from a site at 2,240 m altitude in the equatorial highlands of Burundi. They are presented as a function of depth with the ^{14}C chronology. Pollen counts for 54 more stratigraphic levels have been added to the 78 data published earlier[9] and the average time resolution is therefore a few hundred years. Despite a probable unexplained hiatus in the late glacial and early Holocene, the Kashiru Swamp is one of the few pollen sites in Africa with records extending back 40,000 years.

Except for one study[27], which used a different statistical approach, we are not aware of other quantitative estimates of palaeoprecipitation for tropical Africa. We cannot discuss this record in detail until the mean annual rainfall has been reconstructed for more complete Holocene sequences. The strong aridity trend during the last glacial period needs no further comment as it is well documented by the geological record[10,17,28].

To our knowledge, Figure 2 shows the first continuous palaeotemperature record for the tropics. During the past few thousand years, the average of the reconstructed temperature curve is close to the modern values. The over-estimation of the top-most sample is due to the recent deforestation around the site. The record indicates an average temperature decrease of 4±2 °C for the glacial period between 30 and 13 kyr BP. Moreover, the curve seems to be consistent with the evolution of past vegetation indicated by the same fossil pollen record[9]. Before 30 kyr, the cooling is consistent with the occurrence of montane conifer forest on the Burundi highlands (>2,200 m), which are now tropical rainforest. It also agrees with other pollen data from East Africa[1-3]. The lowest temperature is recorded between 25 and 15 kyr, when the presence of the tree-line and afroalpine grassland at the Kashiru site imply that the mountain vegetation belts had descended by 1,000-1,500 m. The magnitude of cooling in the tropics during the last glacial period is a subject of active discussion. Estimates based on the depression of tree-lines and snow-lines in the high mountains of East Africa, New Guinea and South America indicate a glacial cooling of 5-8 °C[5,7], which appears to be incompatible both with CLIMAP estimates of sea surface temperature and with the temperature in the free atmosphere simulated by the current generation of general circulation models[7]. The Kashiru site is situated at an intermediate elevation. Our estimate of the last glacial cooling, 4±2 °C, is a more conservative value than the estimate of 5-8 °C made at the same site[9], which was based on the assumption that the lapse rate was the same as the present value, 5-6 °C km^{-1}. This is not surprising because displacements of vegetation belts, although difficult to evaluate, depend not only on cooling but also involve precipitation. Our results suggest that the discrepancy between continental and oceanic glacial cooling lies in a failure to distinguish properly between the effects of changes in precipitation and temperature. The curve in Fig. 2 indicates that the optimal time for glacial advance in East Africa (which has not been dated so far) was ~21,500 years ago, when the climate was cold but relatively moist. Our work also indicates that the inferred continental glacial cooling from other tropical regions of the world[29-31] may have been overestimated. Discrepancy between terrestrial temperature decrease for New Guinea and South America and adjacent sea surface temperature might be strongly reduced by further quantitative estimates such as those we have obtained for equatorial Africa. □

TABLE 1 Pollen taxa with the largest loading in the palaeobioclimate operator

Taxa	Statistical distance
Gramineae	+0.38
Anthospermum (Rubiaceae)	+0.26
Ericaceae	+0.22
Podocarpus (Podocarpaceae)	−0.13
Alchemilla (Rosaceae)	−0.15
Combretaceae	−0.16
Hagenia abyssinica (Rosaceae)	−0.16
Polyscias fulva (Araliaceae)	−0.19
Vernonieae (Compositae)	−0.20
Hypericum (Guttiferae)	−0.20
Tubuliflorae (Compositae)	−0.26
Urticaceae	−0.27
Alchornea (Euphorbiaceae)	−0.31
Macaranga (Euphoribiaceae)	−0.34

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Polar ice ablation rates measured using *in situ* cosmogenic ^{14}C

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THE first suggestion that appreciable ^{14}C might be produced *in situ* in polar ice was made by Fireman and Norris[1], who studied ^{14}C in CO_2 extracted from both accumulation and ablation samples. In some ablation samples they observed ^{14}C activities between four and six times higher than those expected due to trapped atmospheric CO_2. Here we report the detection of an unambiguous signal of *in situ* cosmogenic ^{14}C in ice samples from two ablation sites in the Antarctic. The ^{14}C is produced mainly by nuclear spallations of oxygen in ice. The observed concentration of ^{14}C in ablation ice samples is 1–3 × 10^3 atom per g ice—three orders of magnitude higher than expected from the amount of trapped atmospheric CO_2 in this ice. The *in situ* ^{14}C has a unique signature: about 60% exists as ^{14}CO and the remainder as $^{14}CO_2$. This result is consistent with that expected from studies of artificially produced ^{11}C in solid targets. The ^{14}C concentration is found to decrease with depth as expected for *in situ* production. The calculated model ablation rates are found to be 5.8 ± 0.7 and 7.6 ± 0.8 cm yr^{-1} at two sites from the Allan Hills main ice field, in agreement with rates determined by the stake method. Our work indicates that the ^{14}C age of accumulation ice based on trapped (atmospheric) CO_2 would be an underestimate of the true age, if a correction is not made for *in situ* produced $^{14}CO_2$. This can be done easily because the ^{14}C activities of both the CO and CO_2 phases, as well as the trapped CO_2 concentration, can be measured.

The ice samples were collected during the 1987–1988 field season at Allan Hills main ice field by D. Burtner. The sample locations are Cul-de-Sac (76°46′ S; 159°26′ E) and (76°43′ S, 159°22′ E), near station 10 of the triangulation chain by Nishio and Annexstad[2,3]. The elevation[2] of the sites is ~2,000 m. Blocks of ~45 × 45 × 60 cm (depth) were cut and returned whole to the laboratory and stored at −20 °C until use.

Extraction of ^{14}C activity in the ice samples was carried out using a clean vacuum system containing no mercury or teflon. The pumping system consisted of a roughing pump and an oil diffusion pump using a silicone oil of ultra-low vapour pressure. Circulation of gases in the vacuum system was accomplished using a metal-bellows circulating pump (Metal Bellows Corp. Model MB-158) that delivered a pressure head of ~5 torr at pressure of 20 torr.

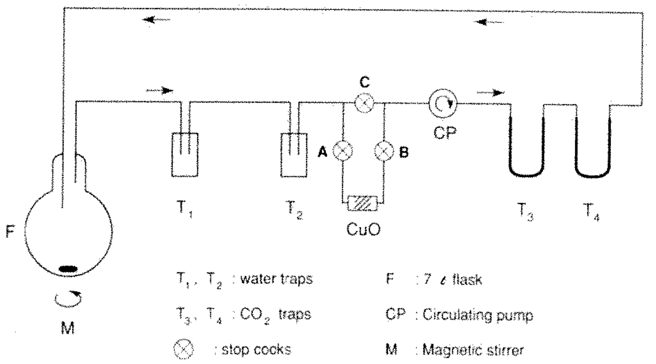

FIG. 1 A schematic of the vacuum line used for extraction of ^{14}CO and $^{14}CO_2$ from ice samples.

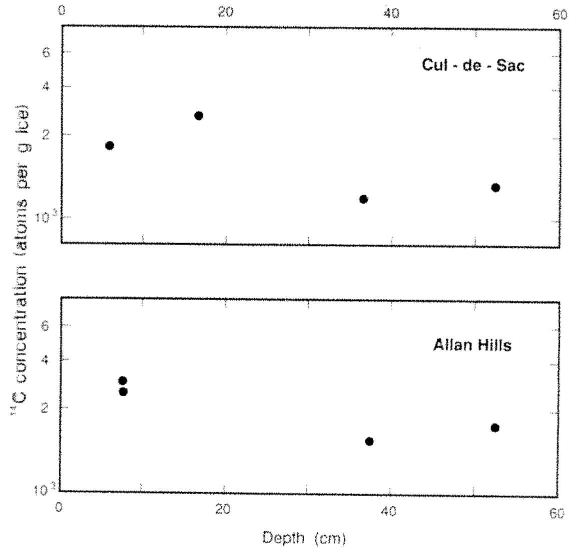

FIG. 2 The measured total ^{14}C concentrations in ice samples, corrected fo line blanks, in the CO_2 and CO_2 phases plotted as a function of depth fo the Allan Hills main ice field Station 10 and the Cul-de-Sac ice samples.

Ice samples were crushed into small pieces and loaded into a 7-l spherical pyrex flask connected to the vacuum system (Fig 1). The flask was cooled in a mixture of salt and ice and then pumped down. Carrier gases of ^{14}C-free CO and CO_2 (1–2 cm^3) were added. The ice sample was then allowed to melt, and the melt water was cooled to 0 °C keeping it well stirred. With stopcocks A and B closed, the circulating pump was then started and the CO_2 frozen out (~2–3 h). The residual gas was passed over conditioned CuO at 600 °C for 1 h with stopcock C closed, and for a further 2 h while the CuO was allowed to cool to <250 °C. The CO_2 gas formed by oxidation of CO (the 'CO fraction') was then frozen out.

Early tests showed that an appreciable amount of the carrier CO_2 was not recovered. This was found to be because the pH of the ice was in the range 6–7, therefore 1M HNO_3 was added lower its pH to ~2. The yields of CO_2 were 30–90%. We assumed that the isotopic ratio of the gas recovered was representative of the total gas and that no exchange between CO_2 and CO had occurred.

The $^{14}C/^{12}C$ ratios of CO and CO_2 samples were determined at the Arizona Accelerator Facility. The results are presented in Table 1 along with the sample details. The concentrations of ^{14}C in CO_2 fraction were calculated assuming that the amount of trapped atmospheric CO_2 was 0.03 cm^3 per kg ice (ref. 4). The calculated values of ^{14}C concentrations have to be corrected for the line blanks. Based on two runs, we determined that the blanks for CO_2 and CO were 2.06 ± 0.14 and 0.32 ± 0.12% of modern carbon respectively. The errors are deduced from counting statistics only. Past experience shows that uncertainties in blank measurements are usually greater than the uncertainties in the counting statistics and are ~25% of the measured values. Here we used 2.0 ± 0.5 and 0.5 ± 0.5% modern for the CO_2 and CO blanks, respectively. As expected if the content in the blank resulted from atmospheric contamination, the CO blank is much smaller than the CO_2 blank. The blanks are much lower than the signals seen for the ice samples. The equivalent measured ^{14}C concentrations are 278 ± 70 and 40 ± 14 ^{14}C atom per g for CO_2 and CO blanks, respectively, assuming that the blank was extracted from 4 kg of ice; the actual quantity of ice varied

TABLE 1 ^{14}C in ablation ice samples

Sample code	Depth (cm)	Weight of ice (kg)	Carrier added (cm^3) CO$_2$	Carrier added (cm^3) CO	^{14}C activity (% modern) CO$_2$	^{14}C activity (% modern) CO	^{14}C concentration (atom per g ice)* CO$_2$	^{14}C concentration (atom per g ice)* CO	Total
Allan Hills Station 10									
ALH 2 (1) T	0–15	5.59	2.11	1.00	14.7 ± 0.7	23.1 ± 0.6	1,490 ± 100	1,160 ± 40	2,655 ± 110
ALH 3 (1) T	0–15	5.22	1.79	1.76	12.5 ± 0.25	20.1 ± 0.33	1,130 ± 60	1,910 ± 60	3,035 ± 85
ALH 2 (2) T	30–45	5.45	2.07	0.98	7.0 ± 0.6	18.7 ± 0.3	590 ± 90	940 ± 30	1,535 ± 100
ALH 2 (2) B	45–60	4.65	2.03	0.97	8.02 ± 0.2	16.3 ± 0.2	810 ± 70	940 ± 30	1,755 ± 80
Cul-de-sac									
CULDE 2 (1) T	0–11.5	4.72	1.84	1.92	8.38 ± 0.24	9.51 ± 0.2	770 ± 70	1,060 ± 60	1,830 ± 90
CULDE 2 (1) B	11.5–22.9	4.45	1.80	1.83	7.89 ± 0.19	14.4 ± 0.2	740 ± 70	1,640 ± 60	2,380 ± 90
CULDE 1 (2) T	30–45	6.25	2.02	0.95	7.28 ± 0.17	15.5 ± 1.6	540 ± 50	650 ± 70	1,190 ± 90
CULDE 1 (2) B	40–60	6.11	1.96	0.92	8.41 ± 0.2	15.5 ± 0.5	650 ± 50	650 ± 30	1,300 ± 65

* Corrected for line blanks of CO$_2$ and CO; see text.

from 4.7–6.1 kg (Table 1). The total blank-corrected ^{14}C concentrations in the ice samples for the two fractions after subtracting the respective line blanks are plotted in Fig. 2.

As seen in Table 1, the repeat measurement of the depth of 0–15 cm at Allan Hills shows a difference in ^{14}C concentrations in the CO fraction, much greater than that expected from quoted errors. Presumably this discrepancy occurred because of incomplete trapping of CO$_2$ during the first phase of the extraction. This would result in some ^{14}C in CO$_2$ being identified as ^{14}C in CO.

The ablation ice in the Allan Hills main ice field is expected to be quite 'old', >100,000 yr based on uranium-series dating method[5,6], which would be consistent with the old terrestrial age of meteorites[7] found in the Allan Hills main ice field. The expected ^{14}C concentration in zero-age ice owing to trapped atmospheric CO$_2$ in the ice samples would be ~950 atom per g ice, taking the typical CO$_2$ concentration to be 0.03 cm^3 CO$_2$ per kg ice (ref. 4). Assuming the age of the ice to be 50,000 yr (a lower limit), we expect the ^{14}C concentration owing to the atmospheric trapped CO$_2$ to be only ~2 atom ^{14}C per g ice. Similarly, we expect the residual *in situ* ^{14}C produced in the ice during its accumulation to be <2 atom ^{14}C per g ice. Thus the signals observed in the CO$_2$ and CO fractions are due nearly entirely to the ^{14}C produced *in situ* in the ice during its outcropping stage. The depth profiles of total ^{14}C concentrations (Fig. 2) are not inconsistent with the expected 1/e depth of ~170 cm (ref. 8) for *in situ* ^{14}C, taking the mean density of ice to be 0.9 g cm^{-3}.

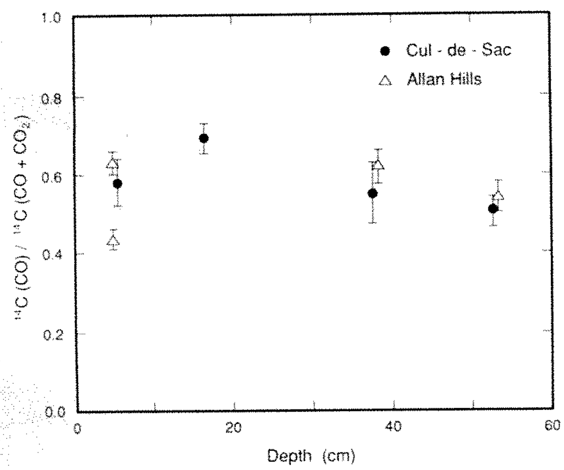

FIG. 3 The measured ratios of concentrations of ^{14}C in the ^{14}CO phase, relative to the total in ^{14}CO$_2$ and ^{14}CO phases, plotted for each of the ice samples studied.

The mean fraction of ^{14}C in CO is 0.57 ± 0.08 compared to the total, for all the measurements (Fig. 3). This should represent primarily the fraction of *in situ* ^{14}C converted to ^{14}CO as the *in situ* produced ^{14}C atom comes to rest[9,10]. In experiments conducted with 'hot' ^{11}C atoms, the ^{11}CO and ^{11}CO$_2$ fractions were found to depend on the nature of the target in which ^{11}C is produced. For solid phase (solid CO$_2$ and NaHCO$_3$ targets), the yields were found to be about equal[9], in good agreement with our result of ~0.6.

The *in situ* production rate of ^{14}C in ice by oxygen spallation has been estimated[8]. At geomagnetic latitudes ⩾60° and 2,000 m altitude this rate is estimated to be 84 atom g^{-1} yr^{-1}. (There is an error in the *in situ* production rate of ^{14}C at 2 km altitude ($\lambda \geq 60°$) given in Lal *et al.*[8]. Using the mean altitude-pressure relationship[11], the revised values for *in situ* ^{14}C production rates owing to spallations in oxygen in ice at 0, 1, 2, 3 and 5 km altitudes and geomagnetic latitudes $\lambda \geq 60°$ are 15, 38, 84, 164 and 480 ^{14}C atom g^{-1} yr^{-1}, respectively.) To this we add a small contribution (<2%) from thermal neutron capture in ^{14}N present in the ice as a constituent of the trapped 'air'. The total *in situ* production rate of ^{14}C in ice at Allan Hills (altitude 2 km and geomagnetic latitude ~60°) is estimated to be 85 atom per g ice yr^{-1}.

The concentration of ^{14}C produced *in situ* in Allan Hills quartz exposed at 2 km was found to be $(8.2 \pm 1) \times 10^5$ atom per g SiO$_2$ (ref. 12). Based on studies of *in situ* ^{10}Be and ^{26}Al in this sample[13], the ^{14}C activity is in secular equilibrium with its production. This yields a production rate of 99 ± 12 atom ^{14}C per g quartz yr^{-1}. This result supports our estimate for production rate of ^{14}C in ice, but suggests that it may be an underestimate by ⩽20%.

We have determined the mean ablation rates for the ice samples based on the uniform ablation model[8]. This model predicts that the steady-state concentration at depth x(cm) from the surface owing to *in situ* production is

$$C(x) = \frac{P_0 \, e^{-\rho x/\Lambda}}{\lambda + \rho a/\Lambda} \qquad (1)$$

where P_0 is the *in situ* production rate of ^{14}C, ρ (g cm^{-3}) is the density of ice, Λ is the absorption mean free path (g cm^{-2}) of cosmic rays in ice, 150 g cm^{-2} (ref. 8), and a is the rate of ablation of ice (cm yr^{-1}). For given measured values of $C(x)$, the equation was solved numerically for the ablation rate a. We obtained 5.8 ± 0.7 and 7.6 ± 0.8 cm yr^{-1} for station 10 and Cul-de-Sac respectively, in good agreement with the value of 3–9 cm yr^{-1} measured[2,3], using stakes, for the Allan Hills main ice field. Annexstad and Schultz[14] obtained an average value of 4.5 cm yr^{-1} for the past 10 yr and W. A. Cassidy (personal communication), a value of ~3.5 cm yr^{-1} for 8-yr average (1978–1986) at a site 2 km northwest of Cul-de-Sac. If the ablation rate has varied significantly in the past, the model ablation rates of ~5.8 and 7.6 cm yr^{-1} would correspond to the rates operative during the period when the ice outcropped from a depth of ~2-3 absorption mean free paths, that is, the past ~50 years. The *in situ* concentration in near-surface ice is attained mostly during this stage.

We did not make any corrections for the atmospheric 'trapped' ^{14}C component because these samples are 'old'. In younger samples, one can determine the absolute amount of the trapped $^{14}CO_2$ component by studying a depth profile of samples. The trapped component is expected to remain constant whereas the *in situ* component will show a depth dependence.

The potential application of *in situ* ^{14}C in accumulation ice have been discussed[8]. Our findings indicate that the *in situ* ^{14}C signal can be seen clearly even in rapidly accumulating ice. For ice accumulating at 2 km altitude in the polar regions, the expected *in situ* ^{14}C concentrations would attain values of $\sim 1.4 \times 10^4/s$ atoms g^{-1}, where s is the accumulation rate (cm yr^{-1}). Because ~60% of the *in situ* ^{14}C is converted to ^{14}CO, one can easily measure accumulation rates up to ~50 cm yr^{-1} in ~3-5 kg ice. □

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Natural occurrence of silicon carbide in a diamondiferous kimberlite from Fuxian

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CONSIDERABLE debate surrounds the existence of silicon carbide in nature, mostly owing to the problem of possible contamination by man-made SiC. Recently, Gurney[1] reviewed reports of rare SiC inclusions in diamonds, and noted that SiC can only be regarded as a probable rather than proven cogenetic mineral. Here we report our observation of clusters of SiC coexisting with diamond in a kimberlite from Fuxian, China. Macrocrysts of α-SiC are overgrown epitaxially by β-SiC, and both polymorphs are structurally well ordered. We have also measured the carbon isotope compositions of SiC and diamonds from Fuxian. We find that SiC is more enriched in ^{12}C than diamond by 20‰ relative to the PDB standard. Isotope fractionation might have occurred through an isotope exchange reaction in a common carbon reservoir. Silicon carbide may thus ultimately provide information on carbon cycling in the Earth's mantle.

Because other workers failed to find the SiC that Moissan claimed to have found in the Canyon Diablo meteorite[2], Mason suggested that Moissan's SiC might have come from the saw used to cut the meteorite. Reports[4,5] of SiC containing data indicative of possible natural origins are rare, and only one paper[6] has described α-SiC in some detail. Data on β-SiC reportedly found in the Green River Formation[7] are very scanty. The only other reported occurrence of β-SiC, described as black spherules in α-SiC (polytype 15R)[8], was based on an X-ray powder diffraction pattern. We believe, however, that this identification cannot be substantiated because the X-ray lines of β-SiC and type 15R overlap completely. The almost 50 samples reviewed by Lyakhovich[9] and described in the original papers resemble artificial SiC (carborundum). Three common sources of contamination are the carborundum fragments used in polishing rock specimens, the drilling equipment bonded with euhedral carborundum crystals used in mining operations, and heavy minerals recovered from rivers polluted with industrial waste[10].

Our specimens came from a kimberlite pipe emplaced in the Sino-Korean Craton near Fuxian, Liaoning (latitude, 39.63° N longitude, 122.00° E). This pipe contains both gem-quality and industrial-grade diamonds. The concentrations of SiC vary from 0 to 0.15% of the host rock—in one instance, we crushed 20 g of kimberlite in an agate mortar, washed the rock fragments to discard the dust-sized particles, and recovered 30 mg of SiC by hand-picking under a binocular microscope. This technique was used throughout our investigation to avoid the use of heavy liquids and contaminations. We used a petrographic microscope to select crystals for analysis by SEM/EDX (scanning electron microscopy/energy-dispersive X-ray) techniques. A Princeton Gamma-Tech Si(Li) windowless detector was used so that the presence of light elements such as C, N, O and F could be verified directly. The solid inclusions and polytype structure were studied using a single-crystal X-ray precession camera. We also measured carbon isotope compositions of 20 mg of α-SiC and five samples of inclusion-free diamonds derived from the same kimberlite pipe.

Large α-SiC crystals (up to 5 mm) are deep blue, but very small crystals often display colour-zoning. Plate-like crystals have a well developed $\{10\bar{1}0\}$ form and are elongated along the a-axis. Rounded resorbed morphologies are common. Crysta aggregates are frequently agglomerated by vesicular milky β-SiC (Fig. 1*a*), and transparent euhedral crystals of β-SiC often occur with the vesicular type (Fig. 1*d*). Intermingled with the β-SiC are spherical grains of quartz occurring as inclusions, protrusions and overgrowths (see Fig. 1*c*, *d*), that are invisible in SEM photographs when totally included by β-SiC (Fig. 1*c*). Many crystal faces are coated by a thin film of iridescent native Si, identified by SEM/EDX.

The zero-level a-axis X-ray precession photograph (Fig. 2*a*) of the dark crystal in Fig. 1*b* shows a well ordered 6H-polytype structure. The light side of the same specimen has a 3C structure (Fig. 2*b*) in which abnormal intensities of the 220 reflections are due to epitaxial growth in which the $[110]_\beta$ and $[10\bar{1}0]_\alpha$ layers are parallel. The 3C structure is also well ordered, as indicated by the discrete diffraction spots. By contrast, commercial SiC is generally composed only of α-SiC that contains numerous stacking faults and multiple polytypes[11].

Crystallographic data of quartz inclusions are derived from X-ray photographs. The reciprocal-lattice net shown in Fig. 2*c* may represent a crystal of either hexagonal or trigonal symmetry as X-ray patterns are centrosymmetric. The lack of vertical and horizontal symmetry planes parallel to c^* and a^*, respectively (Fig. 2*d*), could indicate a $\underline{3}2$ point-group symmetry (trigonal) the symmetry of low quartz (α). But, only a few reflections lack mirror images (owing to extinction or variation in intensity), so the quartz inclusions are, in fact, pseudohexagonal, closely resembling the symmetry of high quartz (β).

As SiC can be synthesized only at very high temperatures and because of its association with diamond-bearing kimberlite

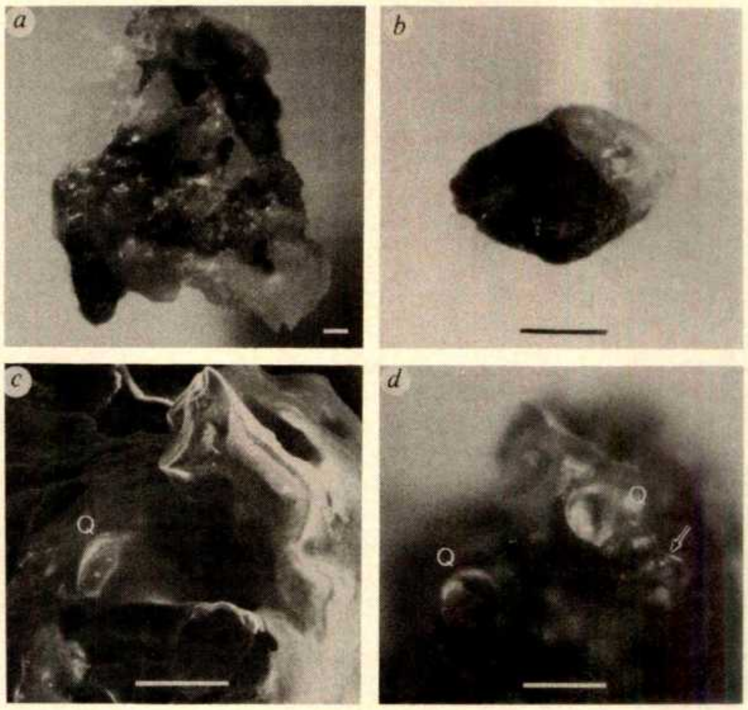

FIG. 1 Scanning electron micrographs of *a*, deep-blue α-SiC crystals overgrown by vesicular milky β-SiC; *b*, epitaxial growth of α-SiC (dark) and β-SiC (light), identified by their X-ray photographs (Fig. 2*a*, *b*); *c*, β-SiC with protruding quartz crystal (Q). Scale bars 100 μm. *d*, Photomicrograph of the same area as *c*, showing two spherical quartz inclusions, which become visible in transmitted light. Arrow indicates euhedral β-SiC crystal. Scale bar 100 μm.

a source region in the Earth's mantle is inferred. Appearance of β-SiC as overgrowths indicates that α-SiC phase must be formed first. Coexisting α-SiC and native Si constitute an assemblage that is stable in environments where the oxygen fugacity is more reduced than the Si–SiO_2 buffer, but we note that geological buffer systems may be highly pressure-dependent[12]. The discovery of a cluster of SiC entrapped in a diamond from Fuxian[13] indicates diamond–SiC paragenesis. Woermann and Rosenhauer[14] estimated theoretically that an assemblage such as diamond + SiC + olivine + orthopyroxene may represent an extremely low redox state. To determine how reduced a chemical environment existed in the mantle when assemblages such as diamond–SiC or diamond–SiC–Si were formed requires a knowledge of the reactants involved in their reaction paths.

Development of vesicular textures in β-SiC requires a gas-rich depressurized environment, which may be encountered during vesiculation of a kimberlitic magma enriched in CO_2. Its rapid ascent[15] may quench and preserve the metastable 3C structure[16] of β-SiC. The appearance of quartz as inclusions and protrusions in β-SiC indicates a cogenetic relation. In the presence of CO_2, native Si associated with α-SiC may undergo the reaction $Si + CO_2 \rightarrow SiC + O_2$ to form β-SiC. In the presence of excess Si, the reaction becomes $2Si + CO_2 \rightarrow SiC + SiO_2$, producing β-SiC and quartz. The pseudohexagonal symmetry of quartz may be the

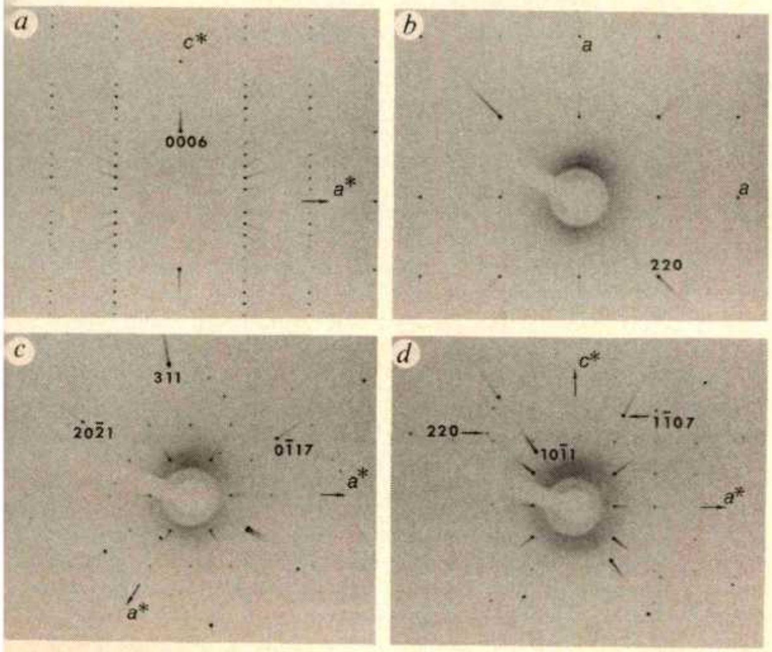

FIG. 2 Zero-level single-crystal X-ray precession photographs. *a*, α-SiC, 6H polytype, *a*-axis. *b*, β-SiC, 3C polytype, *a*-axis. *c*, Spherical quartz inclusion (near centre in Fig. 1*d*), *c*-axis. *d*, Same quartz inclusion, *a*-axis. Indexed extraneous reflections in both *c* and *d* are derived from α- and β-SiC. Mo K_α X-rays (generated using a 20-mA electron beam accelerated to 45,000 V) were used for all photographs. Exposure times, 2–4 days.

TABLE 1 Carbon isotope values of SiC and diamonds from Fuxian

Mineral	Number of crystals	Weight (mg)	Description	$\delta^{13}C$ (‰)*
α-SiC	Numerous	20	blue	−24.0
Diamond	11	12	white, flawless	−2.9
Diamond	19	16	white, deeply etched	−4.5
Diamond	11	10	white, with deformation lamellae	−4.4
Diamond	1	17	pale yellow, flawless	−3.8
Diamond	4	23	pale yellow, flawless	−4.8

Carbon extraction technique: Samples were heated at 1,000 °C for 45 min in circulating O_2 at 1 atm. The combustion tube contained Pt and Cu oxides to oxidize any CO produced during the experiment. 97% of the SiC combusted during the first 5 min, and the remainder combusted within the next 15 min. Yields were better than 99%.

* $\delta^{13}C = [(^{13}C/^{12}C)_{sample}/(^{13}C/^{12}C)_{standard}] - 1$, where the standard was Pee Dee belemnite (PDB).

result of an incomplete displacive transformation, or a quenched high-quartz structure. Crystallization of SiO_2 as quartz instead of coesite indicates a maximum pressure of <20–28 kbar at 1,000–1,200 °C, estimated using equilibrium lines of high quartz/low quartz[17] and quartz/coesite[18] transitions. Under the above pressure and temperature conditions, the stable polymorph of SiO_2 is high quartz, and not tridymite or cristobalite which are stable only at pressures below 10 kbar[19]. Thus, β-SiC must have crystallized in a more oxidizing environment at a depth of 60–90 km where the temperature may be ~1,000 °C, the minimum temperature at which SiC can be synthesized in the laboratory[16].

Results of our carbon isotope analyses are presented in Table 1. The $\delta^{13}C$ values of diamond samples fall in the range, −2.9‰ to −4.8‰ (average = −4.1‰), whereas the value for α-SiC is −24.0‰, indicating a strong ^{12}C enrichment in SiC, ~20‰. Large differences in isotopic compositions have also been observed in diamonds from worldwide localities[20-22]. Hypotheses proposed to account for such isotope heterogeneities include multiple sources of mantle carbon[23], introduction of organic carbon by subduction[24], and extreme fractionation by degassing of the mantle[25], all suggestive of disequilibrium growth of diamonds. As no evidence from diamond research is presently available to justify these hypotheses, we shall discuss an alternative.

Isotopically zoned but non-coated diamonds[23,26] show variations in ^{13}C from core to rim of only about 4‰. We suggest that the 20‰ fractionation observed between SiC and diamond is due to a distillation process: preferential uptake of ^{13}C during the growth of SiC enriched the carbon reservoir, in which the diamond later grew, in ^{12}C. Evidence that the SiC was formed first is provided by the presence of SiC inclusions in diamonds from Fuxian[13]. To explain the preferential uptake of ^{13}C by SiC, we examined the thermodynamic properties (1 atm) of diamond[27] and 6H SiC[28] and found that SiC has much higher enthalpy and entropy than diamond. Deines[29] noted that in an isotope exchange reaction, the solid of lower heat capacity can be correlated with higher vibrational frequency and a stronger tendency to incorporate the heavy isotope. This suggests that SiC should be enriched in ^{13}C and diamond in ^{12}C, as we observe.

In studies of iron meteorites[30,31], systematic ^{12}C enrichment in graphite and ^{12}C depletion in cohenite (iron carbide), in 12‰ fractionation at ~600 °C was observed, and the fractionation was expected to increase with increasing temperature. The ^{13}C–^{12}C systematics observed in our SiC–diamond system is completely analogous. Our isotope data imply that the carbon reservoir in the mantle beneath Fuxian has a $\delta^{13}C$ value between −24‰ and −4‰, and is probably much lower than −5‰, a value derived from isotope analysis of natural diamonds. A more precise estimate would require a knowledge of all fractionation processes and the number of carbon-bearing phases participating in the isotope exchange reaction. ☐

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Rb–Sr dating of sphalerites from Tennessee and the genesis of Mississippi Valley type ore deposits

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MISSISSIPPI Valley type (MVT) ore deposits commonly consist of some combination of lead, zinc and iron sulphides accompanied by barite, fluorite, dolomite and calcite. They are thought to form by fluid expulsion from sedimentary successions[1-3], but their exact origin has remained controversial because of the scarcity of reliable geochronological data. In a recent and widely quoted model the MVT deposits of the central and eastern United States formed as a result of large-scale lateral fluid flow driven by the tectonic squeezing of sedimentary successions during the late Palaeozoic Alleghenian orogeny (330–250 Myr)[4]. Here we report the first direct Rb–Sr dating of sphalerites from an MVT deposit and propose this as a useful geochronological technique for sulphide mineralization with poor age control. Rb–Sr data for sphalerites from Coy mine, East Tennessee, define an isochron age of 377 ± 29 Myr, ruling out any genetic connection with the Alleghenian orogeny. Instead, MVT mineralization in the eastern United States was apparently generated from fluids expelled during thrusting associated with the older Acadian orogeny (380–350 Myr).

The difficulty in isotope dating the MVT deposits is due to

TABLE 1 Rb–Sr data for sphalerites and their fluid inclusions

Sample number		Rb (p.p.m.)	Sr (p.p.m.)	^{87}Rb/^{86}Sr	^{87}Sr/^{86}Sr	Apparent sphalerite-fluid age (Myr)*
C5L	r	0.06686	0.2805	0.6868	0.714635 (69)†	358 ± 21
	l	63.05	3,393	0.05349	0.711404 (85)	
C14I	r	0.2943	1.462	0.5796	0.713633 (19)	306 ± 8
	l	142.1	5,539	0.07387	0.711428 (13)	
C14II	r	0.3364	0.8808	1.101	0.716706 (22)	373 ± 8
	l	150.4	9,984	0.04337	0.711096 (47)	
C15	r	0.2290	1.056	0.6247	0.712408 (20)	150 ± 9
	l	371.1	979.8	0.1090	0.711308 (37)	
C16D	r	0.1707	1.386	0.3549	0.712483 (16)	333 ± 11
	l	47.16	2,403	0.05652	0.711070 (18)	
C16L	r	0.4755	1.858	0.7374	0.714931 (20)	441 ± 9
	l	73.58	1,697	0.1240	0.711079 (21)	
C18	r	0.05042	0.6601	0.2200	0.711761 (16)	233 ± 24
	l	62.10	2,085	0.08579	0.711316 (50)	
C44	r	0.2631	0.4134	1.835	0.720396 (23)	

Rb and Sr were separated using standard ion-exchange procedures[26], and their concentrations measured by isotope dilution using a mixed ^{87}Rb/^{84}Sr spike. In the first column, r and l denote residue and leachate, respectively. Rb and Sr concentrations of fluid inclusions were calculated assuming that the weight fraction of fluid inclusions in the sphalerites was 300 p.p.m. (see text). Isotope measurements were performed using a VG Sector mass spectrometer. During this study, an average ^{87}Sr/^{86}Sr value of NBS 987 Sr standard was 0.710256 (5) (where the number in parentheses indicates the 2σ uncertainty). The decay constant λ ^{87}Rb = 1.42×10^{-11} yr^{-1} was used for the age calculation.

* Uncertainty calculated by combining run precision on Sr isotopic composition with an uncertainty of 1% (2σ) on the ^{87}Rb/^{86}Sr ratio.

† 2σ uncertainty.

the lack of suitable minerals with high or variable parent/daughter ratios. This has led to indirect attempts to determine their age. One approach has been to date apparent resetting from hydrothermal fluids. For example, the Rb–Sr age of glauconites has been used to estimate the age of mineralization in the Viburnum Trend[5] (a 75-km-long belt of MVT mineralization in Missouri), based on the assumption that the formation of the MVT deposit was the last process to affect the isotope system in the glauconites. A second approach is to use model ages. For example, Halliday *et al.*[6] used the large fractionation in Sm/Nd ratios in fluorites from the North Pennine Orefield to place constraints on the timing of mineralization. Similarly, Kesler *et al.*[7] estimated that the mineralization in the Mascot-Jefferson City district in East Tennessee was younger than 395 Myr using a crude basin-evolution model. As with model lead ages, the interpretations are critically dependent on the inferred isotope evolution of the source of the components, which is seldom well defined. There have been very few attempts to date ore constituents directly—one notable exception was that of Lange *et al.*[8] who attempted to date galenas from the Viburnum mine in southeast Missouri using the Rb–Sr method. In addition, Medford *et al.*[9] reported Sr isotope data for sulphide and carbonate minerals from Pine Point mine, Canada, and noted that sphalerite can have a high Rb/Sr ratio which could facilitate its use in isotope dating.

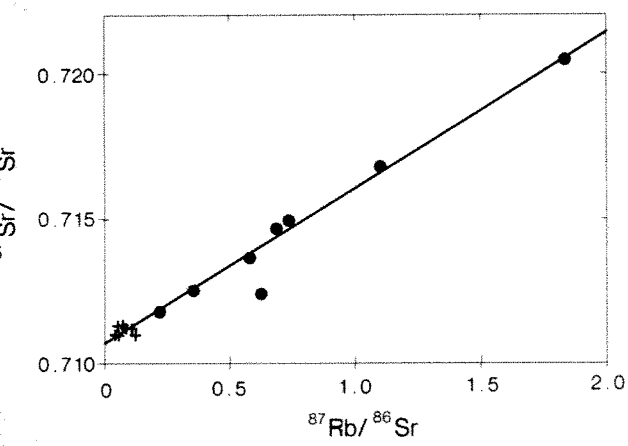

FIG. 1 Rb–Sr isochron of sphalerites from Coy mine. Circles and crosses denote residues and leachates, respectively. Eliminating a point for C15 (see text), the isochron yields an age of 377 ± 29 Myr (mean square of weighted deviates is 62.6).

The deposits in the Mascot-Jefferson City district, East Tennessee, are hosted by the Lower Ordovician Knox group. The top of the Knox group is marked by an extensive Middle Ordovician unconformity, and the ores below occur in collapse breccias formed by karstification[10]. Palaeomagnetic measurements[11] and K–Ar age determinations on clays and K-feldspars in this district[12,13] have been interpreted as indicating large-scale fluid migration in the late Palaeozoic, which led to the view that the Appalachian MVT deposits in East Tennessee were formed by massive movements of fluid expelled from sedimentary basins during the Alleghenian orogeny (330–250 Myr)[4].

Rb–Sr data for eight leached samples of sphalerite, together with seven of their (fluid inclusion) leachates are presented in Table 1 and Fig. 1. For each analysis ~100 mg of hand-picked, washed sphalerite was crushed in deionized water and leached with the water to remove the fluid-inclusion contents. The absolute concentrations of Rb and Sr in the fluid inclusions can only be estimated because of the inability to measure accurately the mass of the inclusions. Haynes *et al.*[14] have reported that gas chromatographic analyses of fluid inclusions in sphalerites from Mascot-Jefferson City district yielded between 3 and 49 μmol per g H_2O (average 13.5 μmol per g) and that the salinities of the fluid inclusions were ~20%. From these data, we estimate that the weight fraction of fluid inclusions in the sphalerites averaged ~300 p.p.m. Rb and Sr concentrations in the fluid inclusions shown in Table 1 were calculated using this value, and are estimated to be accurate to within an order of magnitude. The Rb/Sr ratios, however, are accurate to better than 1%. After removal of the fluid inclusions, the sphalerites were dissolved in hot 6M hydrochloric and concentrated nitric acid (9:1). The sphalerites have low Sr concentrations (<2 p.p.m.), but variable ^{87}Rb/^{86}Sr ratios (0.3–2.0).

The isotope data are plotted on a conventional isochron diagram in Fig. 1 and, with the exception of C15, all the sphalerites show a clear correlation between ^{87}Rb/^{86}Sr ratios and ^{87}Sr/^{86}Sr ratios. The scatter observed probably reflects heterogeneity in the initial Sr isotopic compositions of the sphalerites—such isotope variability for mineralizing solutions is well documented for MVT deposits[8,15]. In addition, the introduction of secondary fluids and the deformation of the ore constituents may also have disturbed the Rb–Sr system. Sample C15 was notably more deformed than the other samples. Ignoring this point, the data for the sphalerites define an apparent isochron age of 377 ± 29 Myr (2σ) with an initial ^{87}Sr/^{86}Sr ratio of 0.7107(3) (2σ uncertainty).

The leachates (fluid inclusions) have lower ^{87}Rb/^{86}Sr ratios (<0.1) than the sphalerites and cluster around the lower end of the isochron in Fig. 1. Calculated apparent Rb–Sr ages for leach–sphalerite pairs are shown in Table 1. The ages obtained are extremely variable, ranging from 150 to 440 Myr. These data indicate that in the relatively large samples required for this study, several types of inclusion fluids (known to exist) were sampled, including secondary fluids not directly related to sphalerite precipitation.

Figure 2 shows a chondrite-normalized rare-earth element (REE) pattern for sample C16D (after removal of the inclusion fluids). As far as we are aware, high-precision REE analyses of sphalerites have not been previously reported. Although the concentrations are low, the pattern is strikingly similar to that of average shales[16]. If the REEs in the sphalerite were derived from the hydrothermal fluids no major fractionation can have occurred during mineralization.

The exact location of the Rb, Sr and REEs in the sphalerites is uncertain. The Rb/Sr ratio is much too high for the components to be derived from carbonate, fluorite or barite inclusions. From the Rb/Sr ratio (0.12) and Sr/Nd ratio (90) of C16D, clay or feldspar are more likely host phases. Scanning electron microscopy (SEM) of the hand-picked sphalerites did not reveal any silicate phases on the micrometre scale except quartz and very rare feldspar on the surface of some of the grains. The K-feldspar seems to predate sphalerite in all samples that we have examined. The sphalerite and feldspar are distributed together in silica-rich alteration zones[13] which are thought to be coeval with mineralization[17]. Our SEM measurements indicate that feldspar inclusions probably constitute $\ll 0.1\%$ of the hand-picked sphalerite, but at least 0.1% is required to explain the Rb and Sr concentrations of C16D. If feldspar is the host of the REEs, the lack of fractionation of Eu relative to a typical crustal pattern (Fig. 2) would require that it grew under non-reducing conditions. We conclude that it is highly unlikely that the Rb–Sr data reflect a dolomite, barite, fluorite or clay component. The Rb, Sr and REEs are most likely hosted in the sphalerite itself or in K-feldspar. These phases are probably cogenetic, therefore the Rb–Sr data should define the age of sphalerite mineralization. If the Rb, Sr and REEs are not held in inclusions, but were sequestered from hydrotherma fluids in minute amounts by the sphalerite, it is not clear how the range in Rb/Sr could have been generated. One possibility is that the composition of the ore fluid evolved with deposition of gangue minerals as a result of different bulk partition coefficients for Rb and Sr. Another possibility is that the variations reflect incomplete mixing of fluids. Both of these seem unlikely because the range of Rb/Sr in the fluid-inclusion contents analysed is relatively restricted (Table 1).

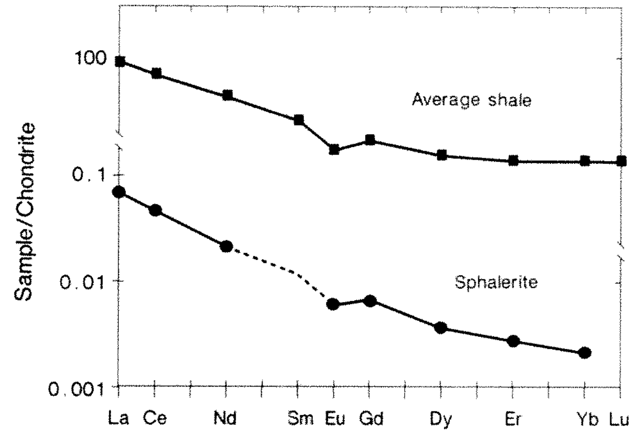

FIG. 2 Chondrite-normalized pattern of a sphalerite, C16D and North American Shale Composite (NASC). The REE abundances in the Leedey chondrite[27] are used to normalize the values. The point for Sm was estimated by interpolating points for Nd and Gd. The pattern of the sphalerite is similar to that of typical crustal material, NASC.

The geologically reasonable isochron age implies that the range in initial Sr isotopic composition in the ore fluids mus have been relatively small. Kesler *et al.*[7] and Kessen *et al.*[1] reported Sr isotope data for MVT deposits in East Tennessee and showed that the gangue minerals that precipitated from the early dolomite-forming fluids had similar Sr isotopic composition to the host carbonates (^{87}Sr/^{86}Sr = 0.7090–0.7092)[7] whereas ^{87}Sr/^{86}Sr ratios for later gangue minerals increased with time up to 0.7122 for paragenetically late calcite. The initial ^{87}Sr/^{86}Sr ratio obtained for sphalerites from the isochron intercept in this study (0.7107) is consistent with this trend, being intermediate between the Sr isotopic ratio for pre-ore and post-ore dolomites Therefore the isotope data are consistent with the idea that the Sr in the sphalerite was sequestered from the ore fluids themselves rather than inherited from unrelated older material in the ore. Furthermore the combined data indicate a relatively small range in Sr isotopic composition of the ore-forming fluids at the time of mineralization.

Our data indicate that mineralization predated the Alleghenian orogeny (330–250 Myr) by ~50 Myr and are therefore inconsistent with the hypothesis that basinal brines tectonically expelled during that event played an important part in ore genesis. Furthermore the alkali feldspar intergrown with sphalerite in these deposits cannot have grown during Alleghenian fluid movements as argued by Hearn *et al.*[13] Although it is generally considered that the thermal effects of the Acadian orogeny (380–350 Myr) were restricted to areas further east than this district[19], it has been shown that there was major thrusting immediately to the southeast of the district at this time[20]. It seems therefore that uplift and deformation in early Devonian resulted in fluid expulsion and the formation of MVT mineralization to the west. MVT deposits located along the Appalachian mountains from Georgia to Newfoundland have many features in common, such as high Zn/(Zn + Pb) ratios and relatively homogeneous Pb and stable isotopic compositions. Among the Appalachian MVT deposits, the mineralization in the St George Group in Newfoundland is similar in stratigraphic setting to that in East Tennessee[10]. ^{40}Ar–^{39}Ar ages of alkali feldspar from MVT ore deposits in western Newfoundland were reported by Hall *et al.*[21]. They concluded that the alkali feldspars were subjected to thermal events at 370–350 Myr and 210 Myr and interpreted the older event as the time of sphalerite mineralization[21]. These data are consistent with the suggestion that Appalachian MVT mineralization was related on a regional scale to the Acadian orogeny.

Oilfield brines are often considered to be suitable ore-forming solutions for MVT deposits[22]. They can have high ^{87}Sr/^{86}Sr ratios owing to interactions with silicate minerals in sedimentary successions. The ratios for several oilfield brines range between 0.709 and 0.735[23,24] and our initial Sr isotopic ratio for the sphalerites and the fluid inclusions falls in this range. Furthermore, Chaudhuri *et al.*[25] have reported Rb/Sr ratios for oilfield brines from Kansas of ~0.02, similar to the ratios found in the fluid inclusions from East Tennessee. Our data are therefore consistent with basin-brine-expulsion models for the fluids that formed both the MVT deposit and the fluid inclusions. ☐

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xperimentally induced life-history evolution in a natural population

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LIFE-HISTORY theory predicts that reduced adult survival will select for earlier maturation and increased reproductive effort; conversely, reduced juvenile survival will select the opposite[1–5]. This is supported by laboratory studies[6–10] and comparative data from natural populations[11–15]. Laboratory studies may support a theory, but cannot assess its importance in natural populations, and comparative studies reveal correlations, not causation[16]. Long-term perturbation experiments on natural populations resolve both problems. Here we report the findings of a long-term study of guppies (*Poecilia reticulata*), in which the predictions of life-history theory are supported. Life-history differences among populations of guppies are closely associated with predator species with which guppies live[13,17–21]. The predators apparently alter age-specific survival because they are size-specific in their choice of prey[21–23]. *Crenicichla alta* (a cichlid), the main predator at one class of localities, preys predominantly on large, sexually mature size classes of guppies[22–24]. *Rivulus hartii* (a killifish), the main predator at another class of localities, preys predominantly on small, immature size classes. Guppies from localities with *Crenicichla* mature at an earlier age, have higher reproductive effort, and have more and smaller offspring per brood than those from localities with just *Rivulus*. These differences are heritable, and correspond with theoretical predictions[17–19]. To prove that predation caused this pattern, we perturbed a natural population of guppies by changing predation against adults to predation against juveniles. This resulted in significant life-history evolution in the predicted direction after 11 years, or 30–60 generations.

In 1976, guppies were transplanted from a site on the Aripo River (Trinidad) with *C. alta* (control site) to a tributary of the Aripo which previously contained *R. hartii* but no guppies (introduction site)[24]. This manipulation released guppies from selective predation on adults and exposed them to selective predation on juveniles. This treatment should favour guppies with delayed maturity and decreased reproductive effort, compared to the control site. The founding population size was 200 adults[24], consisting of equal proportions of males and non-virgin females. As guppies have sperm storage and multiple mating, the effective founding population size was almost certainly greater than 200; founder effects are therefore unlikely. In all

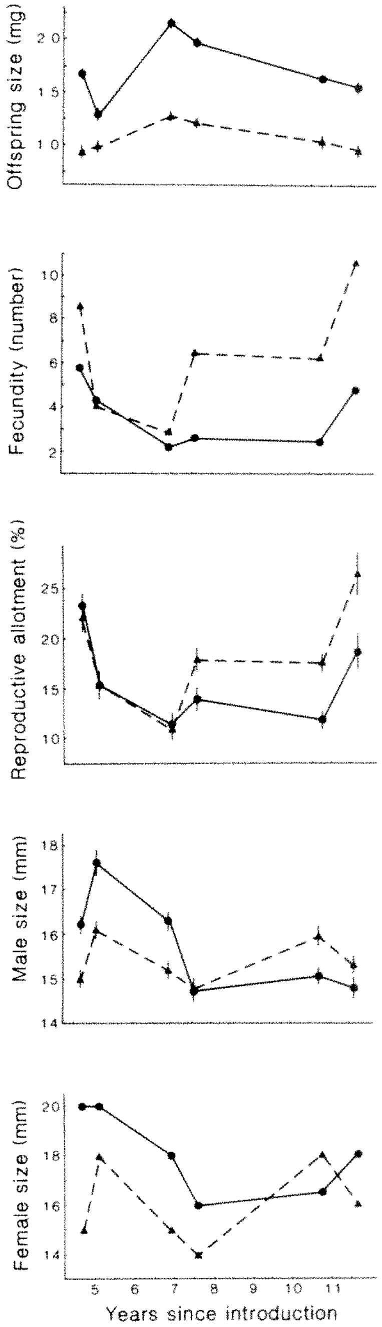

FIG. 1 Life-history phenotypes of guppies from the introduction and control sites. These data are based on wild-caught, field preserved fish. Offspring Size, mean dry weight of developing embryos, corrected for their stage of development and female size. Fecundity, expected number of offspring for a 30 mg (somatic dry weight) female. Reproductive Allotment, per cent of total dry weight that consists of developing embryos, and is corrected for the stage of development of the embryos. Male Size, average size of sexually mature males. Female Size, minimum mm size class in which the majority of females were carrying developing embryos. ●, Introduction site. ▲, Downstream control. All values are least-square means and one standard error from analyses of variance. All statistical comparisons were made within a collection and were executed with the SAS GLM procedure[31].

TABLE 1 Aripo tributary introduction

Life history trait*	Reznick and Bryga (1987)[21] Control (*Crenicichla*)	Reznick and Bryga (1987)[21] Introduction (*Rivulus*)	This study Control (*Crenicichla*)	This study Introduction (*Rivulus*)	Reznick (1982)[17] *Crenicichla*[17]	Reznick (1982)[17] *Rivulus*[17]
Male age at maturity (days)	60.6 (1.8)	72.7 (1.8)†	48.5 (1.2)	58.2 (1.4)†	51.8 (1.1)	58.8 (1.0)†
Male size at maturity (mg-wet)	56.0 (1.4)	62.4 (1.5)†	67.5 (1.2)	76.1 (1.9)†	87.7 (2.8)	99.7 (2.5)†
Female age at first parturition (days)	94.1 (1.8)	95.5 (1.8) (NS)	85.7 (2.2)	92.3 (2.6)‡	71.5 (2.0)	81.9 (1.9)†
Female size at first parturition (mg-wet)	116.5 (3.7)	118.9 (3.7) (NS)	161.5 (6.4)	185.6 (7.5)†	218.0 (8.4)	270.0 (8.2)†
Brood size, litter 1	2.5 (0.2)	3.0 (0.2) (NS)	4.5 (0.4)	3.3 (0.4)‡	5.2 (0.4)	3.2 (0.5)†
Brood size, litter 2	6.3 (0.3)	7.0 (0.3)§	8.1 (0.6)	7.5 (0.7) (NS)	10.9 (0.6)	10.2 (0.8) (NS)
Brood size, litter 3‖	—	—	11.4 (0.8)	11.5 (0.9) (NS)	16.1 (0.9)	16.0 (1.1) (NS)
Offspring size (mg-dry), litter 1	0.91 (0.02)	0.87 (0.02) (NS)	0.87 (0.02)	0.95 (0.02)§	0.84 (0.02)	0.99 (0.03)†
Offspring size, litter 2	0.93 (0.02)	0.86 (0.02)‡	0.90 (0.03)	1.02 (0.04)‡	0.95 (0.02)	1.05 (0.03)‡
Offspring size, litter 3‖	—	—	1.10 (0.03)	1.17 (0.04) (NS)	1.03 (0.03)	1.17 (0.04)†
Interbrood interval (days)	24.9 (0.4)	24.89 (0.4) (NS)	24.5 (0.3)	25.2 (0.3) (NS)	22.8 (0.3)	25.0 (0.03)†
Reproductive effort (%)#	4.0 (0.1)‡	3.9 (0.1) (NS)	22.0 (1.8)	18.5 (2.1) (NS)	25.1 (1.6)	19.2 (1.5)‡

Values are means (s.e.), and represent data from refs 17 and 21, or this study.

NS, not significant.

* Differences in mean values among experiments are attributable to differences in food availability. Ref. 17 had the highest levels, this study was intermediate, and ref. 21 had the lowest levels.

† $P < 0.01$.

‡ $P < 0.05$.

§ $0.05 < P < 0.10$.

‖ Fish were only kept until they produced two litters of young in ref. 21.

# Values for reproductive effort in ref. 21 represent a single estimate made at the end of the experiment; those for the other two studies represen the sum of four consecutive estimates. See ref. 17 for details on the latter analysis.

later visits to the field site, we found guppy populations to be large and widespread. Thus, subsequent genetic drift also seems unlikely.

Two years after the transfer, the phenotypes of females caught from the introduction site differed from those of females from the control site in offspring size and reproductive allotment (embryo weight/total body weight), suggesting a response to changed predation[13]. Six subsequent samples, collected between March 1981 and March 1988, revealed persistent differences between fish from these localities for many life-history variables (Fig. 1). Females from the introduction site produced larger offspring and initiated reproduction at a larger size than those from the control site. They also had smaller brood sizes and smaller reproductive allotments in the four dry-season samples (in (month/year) 3/81, 2/84, 4/87, 3/88), but not in the two wet-season samples (8/81, 6/83). We previously found, in a large-scale survey of many streams, that there was a similar trend towards lower reproductive allotment, lower fecundity and reduced differences between guppies from *Rivulus* and *Crenicichla* localities during the wet season[20]. Mature males from the introduction site were significantly larger than control site males prior to 1984, but smaller thereafter. With the exception of male size, the phenotypes of all variables over 11 years supports the predictions of life-history theory.

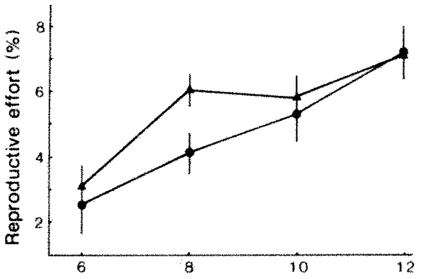

FIG. 2 Reproductive effort in the second generation, laboratory-reared fish at 6, 8, 10 and 12 weeks after the initiation of controlled food availability (following the methods of ref. 19). ▲, Downstream control; ●, Introduction site. Controlled feeding began when the fish were 25 days old. These time intervals correspond to routine changes in food availability. Earlier ages were not included because most females had not yet conceived their first litter. Later ages were not included because many females had already produced their third litter and were no longer in the experiment.

To determine whether these differences were heritable, we collected guppies from both localities in March 1987, then reared their descendants through two generations in a common environment, using the methods of our previous studies[17-19,21]. Rearing fish through two generations in a common environment eliminates environmental influences on the life history; remaining differences are assumed to have a genetic basis. Males and females from the introduction site mature at a significantly later age and larger size than those from the control site (Table 1) they also produce significantly fewer young in the first brood but show no differences in the size of the second or third broods This pattern of increased fecundity early in life corresponds to the results of laboratory selection experiments[10,25] and supports the hypothesis that early-life performance has a stronger influence on fitness than later-life performance[24-26].

Guppies from the introduction site produce larger offspring in all three broods, although the difference is only significant for the second brood. They have lower reproductive effort at 6, 8 and 10 weeks after the initiation of controlled feeding (Fig 2), although the difference was only significant at 8 weeks. The differences in reproductive effort early in life are attributable to the delayed age of first parturition and smaller initial brood sizes in the introduction site guppies. All of these patterns match those observed among fish from two *Crenicichla* and two *Rivulus* sites considered in our earlier study[17] (Table 1). It is therefore clear that the introduction site guppies have evolved genetically new life-history parameters that correspond to the usual differences between these two types of localities.

The genetic differences in life histories reported here are far greater than those observed previously in a similar study[21] (Table 1); although both studies revealed equally strong differences in the life history phenotypes of wild-caught fish. The different results may reflect differences in the duration of the experiments (4 versus 11 years), and may illustrate the time required for a response to selection, arguing strongly for long-term field experiments. As these experiments are duplicates, the general similarity in their results, plus their convergence upon natural populations under the same predation regime, suggest that these are specific responses to natural selection, rather than the results of drift or founder effects.

These results demonstrate significant evolutionary changes in e life histories of introduction-site guppies, which are constent with theoretical predictions. They provide direct experiental field evidence of the importance of predation in moulding fe history evolution in guppies, though of course other factors ay be important[21]. More generally, these results provide vidence that mean differences in age-specific survival will ould the evolution of life-history patterns. The widespread vidence for size-specific predation in other species[27-30] ggests that this could be a common factor in life-history volution. ☐

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ormation of target-specific euronal projections in rganotypic slice cultures rom rat visual cortex

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CHARACTERISTIC feature of the mammalian cortex is that rojection neurons located in distinct cortical layers send their xons to different targets. In visual cortex, cells in layers 2 and project to other cortical areas, whereas cells in layers 5 and 6 roject to subcortical targets such as the lateral geniculate ucleus[1,2]. The proper development of these projections is crucial or correct functioning of the visual system. Here we show that pecific connections are established in an organotypic culture ystem in which rat visual cortex slices are co-cultured with another lice of the visual cortex or with a thalamic slice. The laminar origin and cellular morphology *in vitro* of cortical projections to other cortical regions or to subcortical targets are remarkably similar to those seen *in vivo*. In addition, axons of projecting cells are not restricted to particular pathways, but appear instead to grow directly towards their appropriate target. These observations raise the possibility that chemotropic attraction from the target areas may play an important part in the development of the cortical projection pattern.

Slices of rat visual cortex were co-cultured either with a slice from the lateral thalamus or another slice of visual cortex using a roller-culture technique[3]. We used animals from postnatal day 0 up to postnatal day 2, an age at which the axons of cortical projection neurons in rat have not yet reached their targets[4-6]. The slices were placed side by side less than 1 mm apart on glass coverslips and embedded in a plasma clot (Fig. 1*a*). As reported previously[7], cultures maintained in this manner flatten to one to three cell layers within about 10 days *in vitro*, contain all major cortical cell types and survive for several weeks. In the experiments described here, co-cultures were examined after 8-14 days *in vitro*.

As we were interested in the laminar origin of cortical projection neurons *in vitro*, we studied the layering of the cultured slices in detail. In the rat, the deepest cortical layer (sublayer 6B) forms a band of tightly packed cells lying immediately above the white matter[8]. This cell band was clearly visible in all Nissl-stained slice cultures and formed a sharp border with the white-matter zone (Fig. 1*c*). Usually, the remaining cortical layers could also be identified in cultures maintained for 1-2 weeks and were comparable to those in the normal visual cortex from animals of that age (Fig. 1*b*). The cortical layers of young animals, however, are less distinct than in the adult, and in slice cultures it was sometimes difficult to determine the exact boundaries of every layer. Therefore we used an additional approach to demonstrate the layering in our culture system, taking advantage of the fact that the cortical layers are born in an inside-first outside-last sequence[9,10]. Cells destined for different layers were labelled on their birth by injecting timed pregnant rats with 5-bromodeoxyuridine (BrdU; see Fig. 2 methods). We then prepared slice cultures from 0-2-day-old animals and compared the laminar distribution of labelled cells after 1-3 weeks *in vitro* with the location of labelled cells in littermates of the corresponding age. *In situ*, most cells generated on embryonic day 16 (E16) are located in the deep cortical layers 5 and 6, but cells generated on E18 are concentrated in the upper layers, 2 and 3 (ref. 11; Fig. 2*a* and *c*). In slice cultures, BrdU-labelled cells also formed a distinct band that was only slightly more scattered than *in vivo*. The laminar pattern was very much like the situation *in vivo* at the corresponding age (Fig. 1*d* and Fig. 2). Thus cortical cells in slice cultures are located at their appropriate laminar position.

The origin and cellular morphology of cortical neurons projecting to thalamic slices *in vitro* were determined by placing a small crystal of the lipophilic fluorescent dye DiI in the thalamic explant (see Fig. 3 methods). A micrograph and camera lucida drawing of these cells in a cortical culture are shown in Figs 3 and 4*a* respectively. All but one of the labelled cells were located in the deep cortical layers, precisely where cells projecting to the thalamus are found *in vivo*. In these experiments the thalamus was placed immediately adjacent to the white-matter side of the cortical slice. It is possible that only cells in the deep layers of the cortex, that is, those cells closest to the thalamus, are able to project to this target. To rule out this explanation we placed the thalamus next to the pial surface of the cortical culture, far away from the white matter. Again, axonal innervation of the thalamic explant originated from cells in the deep cortical layers. Cells in the superfical layers, although closer in distance to the thalamic explant, did not innervate this target (Fig. 4*b*). The axons of the deep cortical cells grew directly towards the thalamic explant. In many instances, axon collaterals separate from the main axon at right angles and run horizontally for

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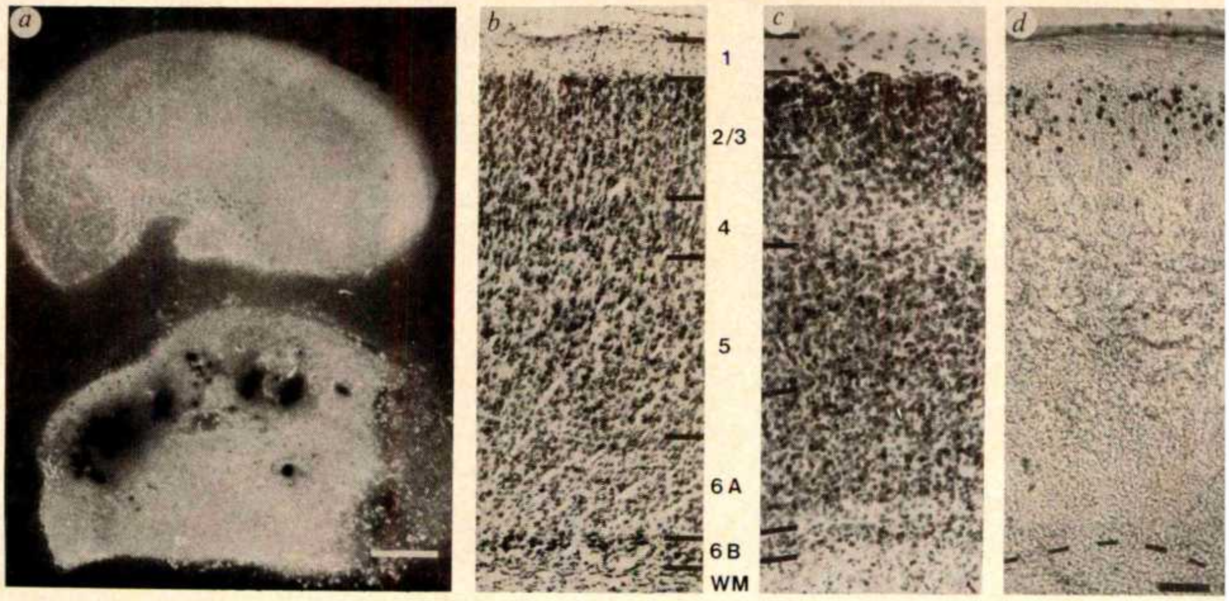

FIG. 1 *a*, Dark-field micrograph of a cortex–thalamus co-culture from a newborn rat fixed after 8 days *in vitro*. The thalamus (below) faces the white-matter side of the cortex (above). The dark spots in the thalamus are DiI crystals that were inserted to trace retrogradely labelled neurons innervating the thalamus *in vitro* (see Fig. 3). Scale bar, 500 μm. *b*, Nissl-stained section from a normal 12-day-old animal *c*, Nissl-stained cortical slice culture from a new-born animal after 12 days *in vitro*. *d*, BrdU-labelled cells in a slice culture from a 1-day-old animal after 10 days *in vitro*. BrdU was injected on E20 (compare with Fig. 2). Scale bar for *b*, *c* and *d*, 100 μm. WM, white matter.

METHODS. Cortical slice cultures were prepared using the roller-culture technique[3] as described previously[7]. In brief, small blocks from the dorsal occipital cortex were cut with a McIlwain tissue chopper at a thickness of 300 μm. Thalamic slices were obtained from a block of tissue containing the dorsal and ventral lateral geniculate nucleus and parts of the surrounding
lateral posterior nucleus. After cutting, the slices were stored for
min at 4 °C in Geys balanced salt solution supplemented with glu
thalamic slice and a cortical slice were then placed adjacent to eac
on a glass coverslip. In some co-cultures the thalamus was place
to the white matter, and in others close to the pial side of the cortic
The tissue sections were then embedded in chicken plasma coagulat
thrombine. The slices were kept in plastic culture tubes (Nunc) and mai
with a medium consisting of 50% Eagle basal medium, 25% Hank's b
salt solution and 25% horse serum containing 0.1 mM glutamine
mg ml^{-1} glucose (all from Gibco). The culture tubes were put in
drum incubator at 36 °C. After 3 days in culture, mitotic inhibitors (5-fl
deoxyuridine, cytosine-beta-D-arabino-furanoside and uridine; conce
10 μM) were added to the medium for 24 h to prevent excessive
of glial cells and fibroblasts.

some distance within the white-matter or in the deep cortical layers. After travelling for up to 1.5 mm, axons then make a second sharp turn and grow through the grey matter to head straight for the thalamus. In control experiments ($n = 24$ cultures) small crystals of DiI were placed near the pial side of the cortical cultures but away from the co-cultured thalamus. When examined after 3–15 days *in vitro*, all cells labelled either through their axon or through their apical dendrites were confined to a restricted region in the culture next to the DiI crystal (distance <500 μm). Therefore, it seems unlikely that
corticothalamic fibres have found their target by initial ra
outgrowth, sending axonal branches in all possible dire
and then eliminating all the branches but the one that
target. Instead, the axons of the deep cortical cells pr
grow directly towards the thalamic explant.

In both sets of experiments, most of the cells innervati
co-cultured thalamus were located in the deep cortical
(40 out of 44 cells in 11 co-cultures). Cells in the sup
layers do not innervate this target. One possible explanati
this is that superfical layer cells are not capable of establ

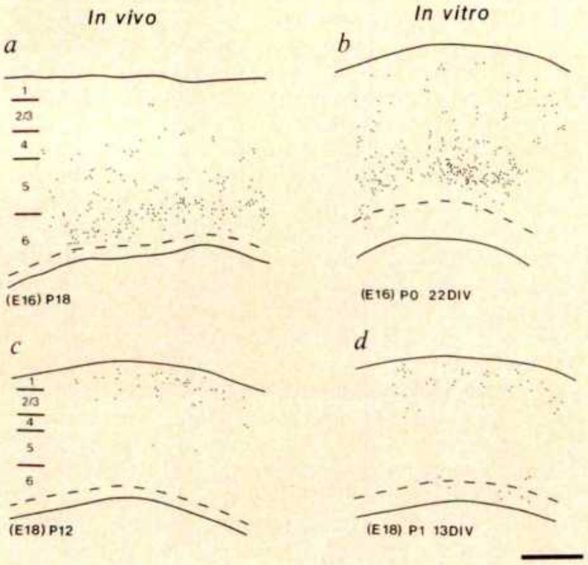

FIG. 2 Distribution of labelled cells in visual cortex *in vivo* and in
slice cultures resulting from an injection of BrdU on different em
days. The cortical layers *in vivo* (numbers on the left) and the
between the grey matter and white matter (dashed lines) were dete
after counterstaining with cresyl violet. Cell counts were made fro
regions as displayed here. *a*, Labelled cells in an 18-day-old anim
BrdU injection on E16; 87% ($n = 254$) of the cells are in the lower
the grey matter. *b*, BrdU-labelled cells in a slice culture prepared
littermate at the day of birth and kept *in vitro* for 22 days; 71% (*n*
of the cells are in the lower half of the grey matter. *c*, BrdU-labelle
in a 12-day-old animal after injection on E18; 93% ($n = 115$) of t
are in the upper quader of the grey matter. *d*, BrdU-labelled cells in a
prepared from a 1-day-old littermate after 13 days *in vitro*; 89% (*n*
of the cells are in the upper quader of the grey matter.

METHODS. Timed, pregnant rats were injected on different embryon
with 200 mg per kg BrdU (Serva, UK). Cells that are generated at t
of injection incorporate BrdU (ref. 35); they were detected with i
histochemical methods and drawn from the microscope with the a
camera lucida.

projections *in vitro*. Alternatively, these cells may not recognize the thalamus as an appropriate target, as, *in vivo*, cells in the upper layers participate in long-range corticocortical projections and do not innervate the thalamus. To examine these possibilities we prepared cultures from two cortical slices placed next to each other. Figure 4*c* shows an example of labelled neurons after application of DiI in the co-cultured cortex. Many retrogradely labelled neurons are found in the superfical layers (126 out of 201 cells in 7 co-cultures), the remaining cells are in the lower layers. The laminar distribution pattern of the labelled cells is very similar to the distribution of corticocortical cells *in vivo*, being mainly situated in the upper layers, but some are also found in layer 5 and in upper layer 6 (refs 12–15).

Not only do the cortical projections established *in vitro* originate specifically from the appropriate layers, but they also seem to be formed by the correct cell type within each layer. First, all projecting neurons have the characteristic morphology of pyramidal cells. Even though several types of intrinsic neurons are known to be present in cortical cultures[7,16], we never observed such neurons with long axonal projections into either the thalamic or the cortical slices. Moreover, there is a close relationship between the pyramidal cell morphology and the efferent target *in vivo*. For example, pyramidal cells in layer 6 that project to the dorsal lateral geniculate nucleus (dLGN) have apical dendrites never reaching higher than layer 3 (ref. 17). Subcortical targets of layer 5 cells include the ventral LGN (vLGN), the lateral posterior (LP) nucleus and the superior colliculus[12,18–20]. *In vivo*, subcortically projecting cells examined so far in layer 5 possess a prominent apical dendrite that always forms a large tuft in layer 1[12,19,20]. They are strikingly different from corticocortically projecting cells in layer 5, whose apical dendrite is short and less branched[12]. Projection neurons *in vitro* exhibit the same variations in dendritic morphology. In the cortical slices co-cultured with the dLGN, vLGN and parts of the LP nucleus (see Fig. 1 methods), projection neurons situated close to the white matter in layer 6 have short apical dendrites. In contrast, many cells in layer 5 in cortex–thalamus co-cultures have a long apical dendrite that extends up towards the pial surface where it often forms an apical tuft. But projection neurons in cortex–cortex co-cultures located in the same laminar position have short apical dendrites.

Since the pioneering studies of Cajal[21] and later those of Sperry[22], several mechanisms have been suggested to explain how specific connections might develop in the vertebrate nervous system. For example, it has been proposed that growing axons are guided by mechanical channels in the neuroepithelium[23], by local chemical cues expressed along the pathway[24], by diffusible factors from intermediate guideposts[25] or directly from the target[26–28]. Relatively few studies have examined the pathfinding mechanisms responsible for establishing efferent projections from the mammalian cerebral cortex. Recently it was proposed that the connections from the cortex to the subcortical targets are laid down by the earliest neurons generated in the cortex, the subplate cells, which act as pioneers for corticofugal axons[29]. There is some controversy as to whether

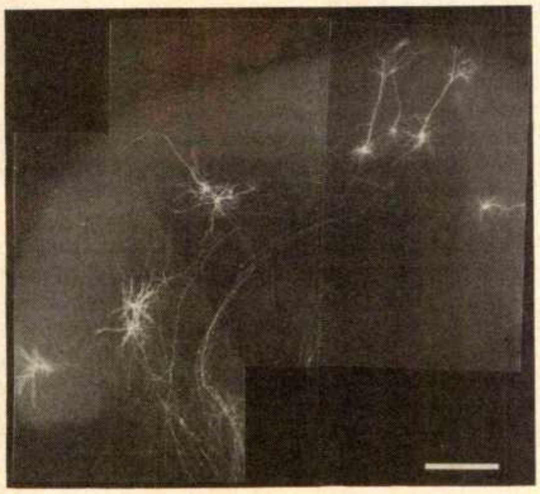

FIG. 3 Fluorescent micrograph of projection neurons in a cortical slice culture retrogradely labelled from the co-cultured thalamus. The pial side is up, the thalamic explant (not shown in the micrograph) faces the white matter zone of the cortical culture. A drawing of these cells is shown in Fig. 4*a*. Scale bar, 500 μm.

METHODS. Cortex–thalamus co-cultures were cultivated for 8–14 days as described in Fig. 1, then fixed in 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4. To visualize the projection neurons, small crystals of DiI (Molecular Probes) were placed in the thalamus[36]. After the co-cultures had been stored in fixative for 6–8 weeks to allow diffusion of the dye within the neuronal membranes, retrogradely labelled cells were viewed with a fluorescent microscope and drawn with the aid of a camera lucida using a ×25 objective. The trajectories of the axons within the thalamus could not be traced as they were masked by the bright fluorescence of the DiI crystals.

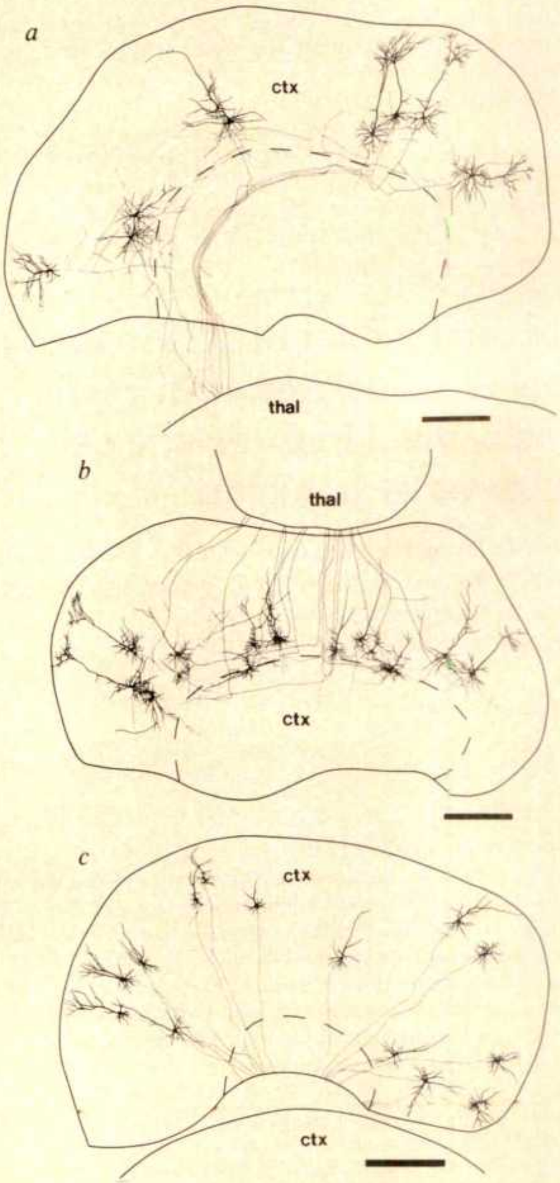

FIG. 4 Camera lucida drawings of cortical projection neurons *in vitro*, retrogradely labelled with DiI from co-cultured thalamus (thal) or cortex (ctx). The thalamus is placed towards the white matter (*a*) or close to the pial surface of the cortical slice (*b*). *c*, Projection neurons in a cortex–cortex co-culture. The borders between the grey and white matter (dashed lines) were determined after the cultures were stained with cresyl violet. Scale bars, 500 μm.

subplate cells are transitory cells that disappear after birth[30,31] or whether they remain present throughout the animal's life[8]. Thus, it is not clear whether these cells are present at all in our cultures taken from postnatal animals. Our retrogradely stained projection neurons did not, however, show the characteristic morphological features of subplate cells[8,32,33]. It thus seems that the projection of subplate cells is not necessary for the establishment of cortical projections *in vitro*. In some, but not all co-cultures, we also observed ingrowing thalamic afferents into the cortical cultures (N.N., V. Staiger, T.B. and J.B., manuscript in preparation). But in all co-cultures shown in Fig. 4, no thalamic afferents were detected despite the intense labelling of many corticofugal cells. It is therefore unlikely that the cortical projections we observed are guided by, or dependent on, ingrowing afferents.

Recent experiments *in vitro* have shown that thalamic explants placed close to the white-matter of cortical explants are innervated by cortical cells from the deep cortical layers[34]. These findings are confirmed and extended by our data, showing that the laminar position and cellular morphology of cells giving rise to cortical connections established *in vitro* are specific and target-dependent. Furthermore, the present study demonstrates that ectopically positioned projection sites are innervated only by a specific subset of cortical projection neurons, even if their axons have to travel for long distances. The axonal trajectories were always oriented towards the target and not restricted to particular pathways. This suggests that some form of chemotropic attraction may play a part in the formation of specific connections between cortical neurons and their targets. ☐

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Structural model of ATP-binding proteins associated with cystic fibrosis, multidrug resistance and bacterial transport

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THE ATP-binding cassette (ABC) superfamily of transport systems now includes over thirty proteins that share extensive sequence similarity and domain organization (reviewed in refs 1–3). This superfamily includes the well characterized periplasmic binding protein-dependent uptake systems of prokaryotes, bacterial exporters, and eukaryotic proteins including the P-glycoprotein associated with multidrug resistance in tumours (MDR), the STE6 gene product that mediates export of yeast a-factor mating pheromone, pfMDR that is implicated in chloroquine resistance of the malarial parasite, and the product of the cystic fibrosis gene (CFTR). Here we present a tertiary structure model of the ATP-binding cassettes characteristic of this class of transport system, based on similarities between the predicted secondary structures of members of this family and the previously determined structure of adenylate kinase. This model has implications for both the molecular basis of transport and cystic fibrosis and provides a framework for further experimentation.

Transporters in this superfamily of transport proteins each seem to be relatively specific for a single substrate (or group of related substrates) which can be a small molecule such as an amino acid or sugar, or a large molecule such as a polysaccharide or protein. In general, each transporter consists of four distinct domains that may be fused in a variety of ways (Fig. 1). The ATP-binding domains, which provide the principal distinguishing feature of this family of transport proteins, share about 30% sequence identity over a cassette of about 200 amino acids (Fig. 2) and include the two short ATP-binding motifs present in the nucleotide-binding pockets of many proteins (Walker motifs; refs 4, 5). Proteins containing these ATP-binding cassettes (some of which are not associated with transport; for example, UvrA, a DNA repair enzyme) are designated ABC proteins. It is now clear that hydrolysis of ATP by these cassettes provides the energy requirement for substrate accumulation[5-10]. As there is no evidence for subunit phosphorylation (ref. 11; C.F.H., unpublished data), this probably involves a conformational change that is transmitted to the transmembrane domains. To understand the mechanism of energy coupling, structural data are required and, in the absence of experimental data, we have derived a tertiary structure model of the ATP-binding cassette.

As a first step in modelling, potential secondary structures were predicted from the primary sequences of ABC-proteins using the Chou-Fasman algorithm[12]. Confidence in the predicted consensus secondary structures (Fig. 2) was enhanced by the availability of so many closely related proteins. Folding this linear secondary structure map into a tertiary structure must be based on an experimentally determined structure. Comparison of the secondary structure map of the ABC proteins with that of several ATP-binding proteins whose structures have been determined (adenylate kinase (ADK); phosphoglycerate kinase (PGK); phosphofructokinase (PFK); hexokinase (HK), and pyruvate kinase (PK); refs 13–16, and T. N. Bryant, P. J. Shaw, N. P. Walker, P. L. Wendell and H. C. Watson, unpublished results), showed remarkable similarity to that of adenylate kinase

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(Fig. 2). The most important difference was an additional loop predicted for the ABC proteins (loop 2) inserted between α-helices C and E of ADK. Assuming the ABC proteins fold in a similar manner to ADK[13], this loop would be inserted at the surface of the protein and would not be expected to perturb the overall structure. Insertion or deletion within loops does not necessarily perturb the structure of a nucleotide-binding fold[17].

A tertiary structure of the ABC cassette was built in two stages using the protein modelling facilities within QUANTA and CHARMm[18]. First, constraints were applied in regions of predicted α-helix or β-sheet (Fig. 2) to give an extended α-carbon structure in which no turns were built. This structure was then folded, adjusting torsion angles in the regions predicted to be turns, to place the α-helices and β-sheets in the same configurations as found in ADK. The predicted secondary structure elements of the ABC proteins could readily be accommodated within the ADK fold without imposing any abnormal constraints on the protein. In contrast, it was not possible to fold the ABC proteins into structures similar to those of PGK, PFK, PK or HK, given the lengths of loops between elements of secondary structure.

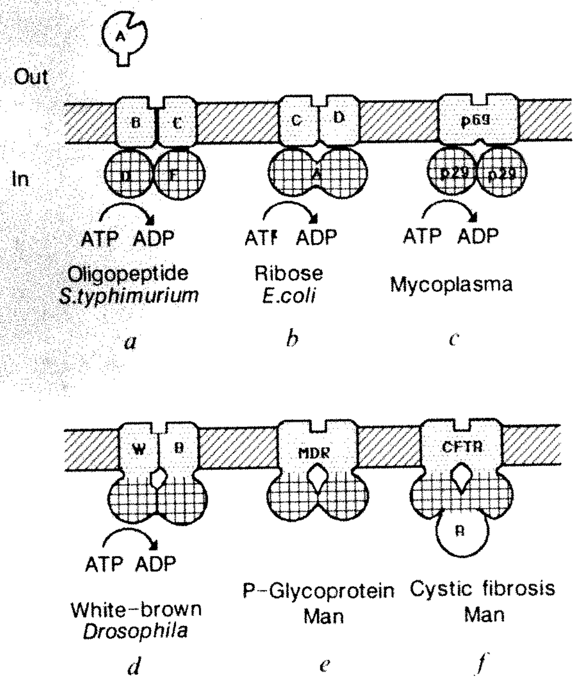

FIG. 1 The organization of ATP-binding cassette (ABC) transporters. The basic transporter requires four distinct domains. Two of these are highly hydrophobic, integral membrane proteins—each of which spans the membrane five or six times by putative α-helices—and are believed to bind the substrate and mediate its passage across the lipid bilayer (stippled). The other two domains couple ATP hydrolysis to transport and are associated with the cytoplasmic face of the membrane (hatched). *a*, In many bacterial systems (for example, the oligopeptide permease of *Salmonella typhimurium*; ref. 29) the four domains are encoded as separate polypeptides (B, C, D, F). These bacterial uptake systems also require the function of a periplasmic substrate-binding protein (A, OppA). However, as mutants which function in the absence of this protein can be isolated[30], and as exporters and eukaryotic systems seem to lack an equivalent component, the periplasmic proteins are probably best considered as accessory components and not essential to the mechanisms of transport across the bilayer *per se*. In other systems the domains may be fused into multidomain polypeptides in various ways. *b*, In the ribose system of *Escherischia coli* the two ATP-binding subunits are fused into a single polypeptide (A)[31]. *c*, In the Mycoplasma p69 system the two hydrophobic domains are fused into a single polypeptide[32]. *d*, The *Drosophila white* and *brown* loci[33] and the HlyB haemolysin export system of *E. coli*[34] each consist of one hydrophobic domain fused to an ATP-binding domain. *e*, *f*, In each of the other eukaryotic systems characterized so far—including MDR (ref. 35), STE6 (refs 36, 37) and CFTR (ref. 23)—all four domains are fused into a single polypeptide. CFTR also has a fifth domain—the R-domain[23]—which probably serves a regulatory role. Finally, certain transporter systems appear to lack domains. For example, the histidine and maltose transporters only have a single gene encoding an ATP-binding domain[38,39], and the HlyB protein and ProU glycine betaine transporters only appear to have one ATP-binding and one hydrophobic domain[34,40]. It seems likely that, in such cases, the polypeptides interact as homodimers to provide the four basic domains seen in other systems[41].

In the second stage of modelling, this crudely folded α-carbon model was made to resemble the known ADK structure more closely, using the homology building function of QUANTA to reposition each amino acid in each of the conserved secondary structure elements in the same position in the structure occupied by the equivalent residue in ADK (Fig. 2). Only four short stretches of sequence, designated loops 1, 2, 3 and 4 (Fig. 2), had no direct counterparts in ADK and these were modelled as described in the legend to Fig. 3. Only then were amino acid side chains added to the α-carbon model and energy minimization—constraining main chain positions—used to relieve any unrealistic torsions, poor geometry or contacts between amino acids to give an energetically feasible structure (Fig. 3). Finally, as atomic coordinates for ATP bound to ADK are not available, ATP was docked onto the ADK structure to satisfy NMR nuclear Overhauser effect criteria[19] (Fig. 3) and then added to the ABC model by analogy with its location within ADK.

Our model of the ATP-binding cassette satisfies the general rules of protein folding, although other structures might also fulfill these criteria. However, several lines of evidence support the underlying assumption that the ABC proteins fold in a similar manner to ADK. First, ADK was the only ATP-binding protein that could provide a framework on which the ABC proteins could be modelled without introducing unacceptable constraints. Second, the model was initially derived without considering the nature of the amino-acid side chains (except in so far as they influenced potential secondary structures). When subsequently added to the model, the amino-acid side chains were found to be positioned in a manner consistent with an actual protein structure. Thus, the hydrophobic β-sheets of the Rossmann fold[20] were maintained (Fig. 3*c*), there were no inappropriately buried hydrophilic residues, and patches of hydrophilic residues were confined to the surface of the structure. Furthermore, the model predicts a cleft of dimensions suitable for binding ATP; no unacceptable amino-acid side chains are exposed within the cleft and the two Walker motifs are appropriately positioned. Finally, regions of the ABC proteins that are hypervariable in sequence are exposed at the surface of the structure, and insertions or deletions of amino acids between ABC proteins occur in regions predicted to form loops and would, therefore, not be expected to affect the core structure[17].

The most important differences between the model of the ABC domain and the known structure of ADK are loops 2 and 3. Loop 2 has no equivalent in ADK and, given where it is inserted at the surface of the structure, it seems unlikely to be involved in ATP-binding and hydrolysis; the model implies that loop 2 serves a role specific to the ABC proteins. The fact that loop 3 is so highly conserved in ABC proteins implies that it, too, serves an ABC-specific function rather than being involved in ATP-binding or hydrolysis. Importantly, the regions of ADK equivalent to loops 2 and 3 are those that undergo the greatest conformational changes on binding ATP[21,22]. We suggest that loops 2 and 3 may be responsible for coupling ATP-dependent conformational changes to the transport process, presumably by an interaction with the hydrophobic membrane domains of the transport systems. The hydrophobicity of loop 2 is consistent with such a role. Similarly, α-helix E, which links loop 2 to the main body of the protein, is a highly conserved structure despite considerable sequence variation between the ABC proteins, consistent with a role in transducing conformational changes to loop 2.

Intriguingly, loops 2 and 3 are precisely where the published cystic fibrosis defects lie. About 70% of Caucasian cystic fibrosis (CF) patients have phenylalanine 508 deleted from loop 2 of the first ATP-binding domain of the CF protein (CFTR) (ref. 23). The adjacent amino acid, isoleucine 507, is deleted in some other patients (M. Super, personal communication); a cluster of mutations in loop 3 has been reported by Cutting *et al.*[24]. Significantly, temperature-sensitive mutations selected in the related FtsE cell division protein of *E. coli*[25] are all located in loops 2 and 3 (D.R.G., unpublished data). The fact that CF mutations are clustered in loops 2 and 3 argues against the suggestion[23] that CF patients are defective in binding or hydrolysing ATP. Furthermore, any mutation that prevents ATP binding would inactivate the protein and have the same phenotype as a null mutation; although frame-shift and nonsense mutations in the CF gene have been identified[24,26], spontaneous mutations leading to CF arise infrequently, suggesting that individuals homozygous for null mutations are either not viable or do not exhibit a strong phenotype. We therefore suggest that CF is a consequence of a specific alteration in the function of the CFTR protein, for example its substrate specificity, rather than simply a loss of CFTR activity. Specifically, CF mutations may alter interactions between the ATP-binding cassette and the membrane-associated domains of the transport system. This model would predict that other CF mutations (excluding null mutations) will alter loops 2 or 3 of either of the two ATP-binding domains of the CFTR protein, modify sequences in the ATP-binding domains that transmit conformational changes to loop 2, or affect regions of the hydrophobic membrane domains with which the ATP-binding domains interact.

It is well established that CF patients have altered chloride-channel activity (reviewed in ref. 27). On the basis of our understanding of ABC transporters, it seems unlikely that the CFTR protein is the chloride channel itself. First, ABC-transporters accumulate substrates against concentration gradients, requiring ATP hydrolysis, but the chloride channels do not. Second, ABC transporters are unidirectional (either import or export), whereas the chloride channel can operate in either

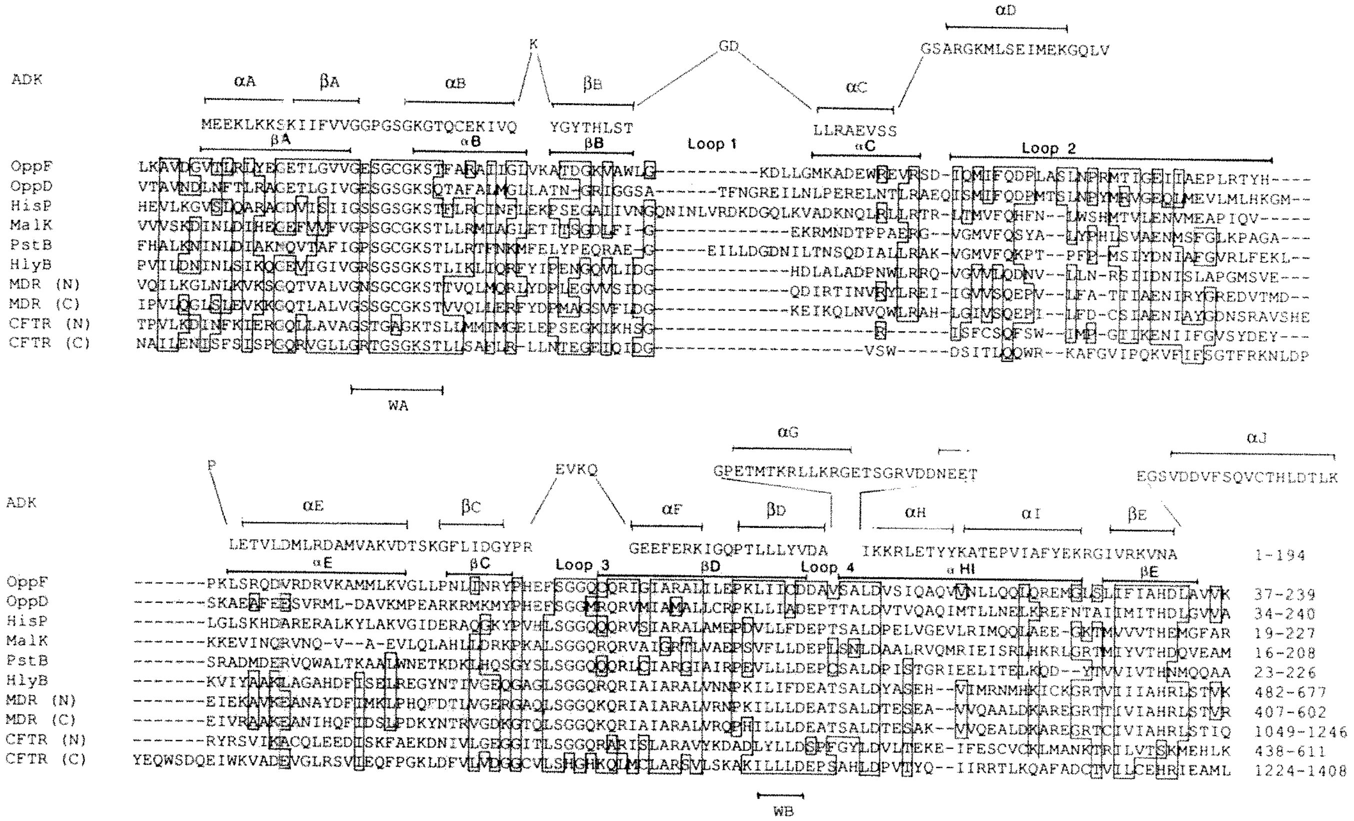
αD
K
GD
GSARGKMLSEIMEKGQLV
ADK
αA
βA
αB
βB
αC
MEEKLKKSKIIFVVGGPGSGKGTQCEKIVQ
YGYTHLST
LLRAEVSS
βA
αB
βB
Loop 1
αC
Loop 2
OppF LKAVDGVTLRLYEGETLGVVGESGCGKSTFARAIIGLVKATDGKVAWLG----------KDLLGMKADEWREVRSD-IQMIFQDPLASLNPRMTIGEIIAEPLRTYH----
OppD VTAVNDLNFTLRAGETLGIVGESGSGKSQTAFALMGLLATN-GRIGGSA------TFNGREILNLPERELNTLRAEQISMIFQDPMTSLNPYMRVGEQLMEVLMLHKGM--
HisP HEVLKGVSLQARAGDVISIIGSSGSGKSTFLRCINFLEKPSEGAIIVNGQNINLVRDKDGQLKVADKNQLRLLRTR-LTMVFQHFN--LWSHMTVLENVMEAPIQV-----
MalK VVVSKDINLDIHEGEFVVFVGPSGCGKSTLLRMIAGLETITSGDLFI-G--------------EKRMNDTPPAERG--VGMVFQSYA--LYPHLSVAENMSFGLKPAGA---
PstB FHALKNINLDIAKNQVTAFIGPSGCGKSTLLRTFNKMFELYPEQRAE-G-----EILLDGDNILTNSQDIALLRAK-VGMVFQKPT--PFP-MSIYDNIAFGVRLFEKL--
HlyB PVILDNINLSIKQGEVIGIVGRSGSGKSTLIKLIQRFYIPENGQVLIDG-------------HDLALADPNWLRRQ-VGVVLQDNV--LLN-RSIIDNISLAPGMSVE---
MDR (N) VQILKGLNLKVKSGQTVALVGNSGCGKSTTVQLMQRLYDPLEGVVSIDG-------------QDIRTINVRYLREI-IGVVSQEPV--LFA-TTIAENIRYGREDVTMD--
MDR (C) IPVLQGLSLEVKKGQTLALVGSSGCGKSTVVQLLERFYDPMAGSVFLDG-------------KEIKQLNVQWLRAH-LGIVSQEPI--LFD-CSIAENIAYGDNSRAVSHE
CFTR (N) TPVLKDINFKIERGQLLAVAGSTGAGKTSLLMMIMGELEPSEGKIKHSG--------------------R------ISFCSQFSW--IMP-GTIKENIIFGVSYDEY---
CFTR (C) NAILENISFSISPGQRVGLLGRTGSGKSTLLSAFLR-LLNTEGEIQIDG---------------------VSW-----DSITLQQWR--KAFGVIPQKVFIFSGTFRKNLDP
WA
αG
αJ
P
EVKQ
GPETMTKRLLKRGETSGRVDDNEET
EGSVDDVFSQVCTHLDTLK
ADK
αE
βC
αF
βD
αH
αI
βE
LETVLDMLRDAMVAKVDTSKGFLIDGYPR
GEEFERKIGQPTLLLYVDA
IKKRLETYYKATEPVIAFYEKRGIVRKVNA
1-194
αE
βC
Loop 3
βD
Loop 4
α HI
βE
OppF -------PKLSRQDVRDRVKAMMLKVGLLPNLINRYPHEFSGGQCQRIGIARALILEPKLIICDDAVSALDVSIQAQVVNLLQQLQREMGLSLIFIAHDLAVVK 37-239
OppD -------SKAEAFEESVRML-DAVKMPEARKRMKMYPHEFSGGMRQRVMIAMALLCRPKLLIADEPTTALDVTVQAQIMTLLNELKREFNTAIIMITHDLGVVA 34-240
HisP -------LGLSKHDARERALKYLAKVGIDERAQGKYPVHLSGGQQQRVSIARALAMEPDVLLFDEPTSALDPELVGEVLRIMQQLAEE-GKTMVVVTHEMGFAR 19-227
MalK -------KKEVINQRVNQ-V--A-EVLQLAHLLDRKPKALSGGQRQRVAIGRTLVAEPSVFLLDEPLSNLDAALRVQMRIEISRLHKRLGRTMIYVTHDQVEAM 16-208
PstB -------SRADMDERVQWALTKAALWNETKDKLHQSGYSLSGGQQQRLCIARGIAIRPEVLLLDEPCSALDPISTGRIEELITELKQD--YTVVIVTHNMQQAA 23-226
HlyB -------KVIYAAKLAGAHDFISELREGYNTIVGEQGAGLSGGQRQRIAIARALVNNPKILIFDEATSALDYASEH--VIMRNMHKICKGRTVIIIAHRLSTVK 482-677
MDR (N) -------EIEKAVKEANAYDFIMKLPHQFDTLVGERGAQLSGGQKQRIAIARALVRNPKILLLDEATSALDTESEA--VVQAALDKAREGRTTIVIAHRLSTVR 407-602
MDR (C) -------EIVRAAKEANIHQFIDSLPDKYNTRVGDKGTQLSGGQKQRIAIARALVRQPHILLLDEATSALDTESAK--VVQEALDKAREGRTCIVIAHRLSTIQ 1049-1246
CFTR (N) -------RYRSVIKACQLEEDISKFAEKDNIVLGEGGITLSGGQRARISLARAVYKDADLYLLDSPFGYLDVLTEKE-IFESCVCKLMANKTRILVTSKMEHLK 438-611
CFTR (C) YEQWSDQEIWKVADEVGLRSVIEQFPGKLDFVLVDGGCVLSHGHKQLMCLARSVLSKAKILLLDEPSAHLDPVTYQ--IIRRTLKQAFADCTVILCEHRIEAML 1224-1408
WB

FIG. 2 Primary and predicted secondary structures of ABC-proteins. A number of ABC proteins are aligned to show sequence conservation (shaded). The locations of the predicted secondary structure motifs used in modelling are indicated. Each element is named according to the equivalent element in ADK. The predictions were generated using the Chou-Fasman algorithm and modified in light of a consensus derived from multiple predictions on other ABC proteins. Although such algorithms are not entirely reliable, for such a large family of related proteins confidence is increased for consensus secondary structures that are predicted in the same position in each protein even where the primary sequences differ. In addition, the same algorithm was used as a control to predict secondary structure elements of ADK (porcine); although ADK was part of the database used in formulating the secondary structure prediction methods, it was encouraging that the predicted structures were consistent with those determined by crystallography[13]. Many ABC proteins include amino acids at the N- and C-termini, outwith the conserved ATP-binding cassette[41]. It is reasonable to assume that these amino acids have no important effect on the structure of the ATP-binding cassette, which must fold in a context-independent fashion, and they were excluded from the modelling. The amino-acid residues modelled are those shown in the figure, numbered as in the complete primary sequences of the proteins. The ADK sequence is shown above the alignment of the ABC proteins. The ADK structural elements indicated are those determined by crystallography[13]. The ADK sequence is aligned with that of the ABC proteins, although this alignment is based on secondary structure similarities and not on primary sequence conservation; apart from the Walker motifs[4], which are labelled WA and WB, ADK shares little primary sequence similarity with the ABC proteins. The lower line of ADK sequence represents residues which, in the final model, were replaced directly by aligned residues from the ABC proteins. Numbering of the ADK amino acids is according to the AK1 designation[21]. Residues in the upper line have no direct counterpart in the model of the ABC proteins. The modelling of these loops is discussed in the text and in the legend to Fig. 3. Residue Phe 508, deleted in many CF patients[23], is indicated. The amino-acid sequences were from the following sources: OppD and OppF (ref. 29); HisP (ref. 38); MalK (ref. 39); HlyB (ref. 34); PstB (ref. 42); Mdr (ref. 43); CFTR (ref. 23).

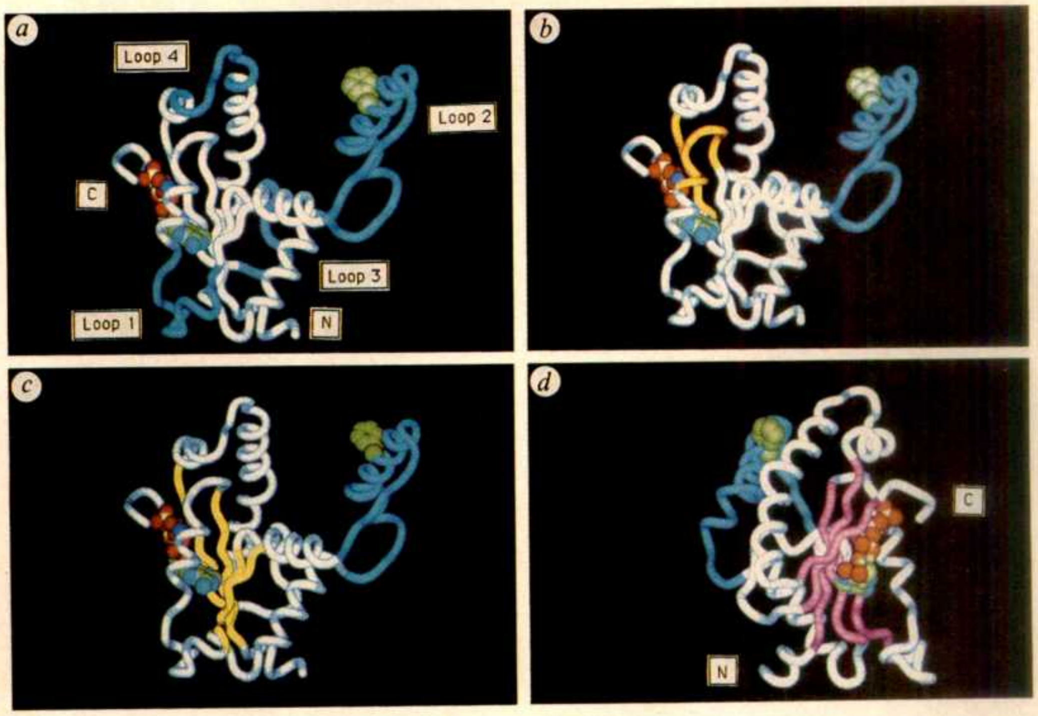

FIG. 3 Tertiary structure model of the ABC cassette. The four panels show different views of the model structure with different motifs highlighted. The core of the model, based on the structure of ADK, was derived as described in the text. Loops 1, 2, 3 and 4 have no direct counterparts in ADK and were modelled separately. Loops 1, 3 and 4 were comparatively easy to model as they had simply to be shortened or enlarged compared with the equivalent loops in ADK, though maintaining the orientation of the flanking secondary structure motifs. The modelling of loop 2 was more difficult as there is no analogue in ADK. Although the point at which this loop emerges from the core structure is defined, the conformation adopted by the loop itself cannot be predicted with any certainty and must be viewed in this light. The loops were selected from a fragment database essentially as described[44] and docked into the main body of the structure by fragment movement and appropriate adjustment of torsion angles. The residues around the insertion of the loops were standardized using 200–400 steps of steepest descent and 100–200 steps of ABNR minimization. Quanta software provided by Polygen Corporation, Waltham, Massachusetts, USA. The locations of residue Phe 508 in the N-terminal CF cassette, and the bound ATP molecule, are shown in 'space-filling' format. ADK has two nucleotide-binding sites, one for AMP and one for ATP[21]. However, these have not been defined at an atomic level. We assume that only one nucleotide is bound by the ABC proteins and that this is likely to be at the equivalent of the 'AMP' site of ADK[21]. In particular, sequences in ADK that are thought to interact with the bound nucleotide in the 'AMP' site[13,21] are conserved in the ABC proteins. In contrast, amino acids (for example, Asp 93, Arg 44) in the 'ATP' site of ADK are absent from the ABC proteins. Furthermore, this site in ADK seems to be able to bind ATP[19]. Therefore, ATP was docked into the 'AMP' site of ADK based on NOE criteria obtained for ATP bound at this site[19]. Because a conformational change occurs in ADK on binding ATP[21] it is not possible to be definitive about the precise location of the ATP, but the position shown is consistent with the proposed interactions between ATP and the Walker motif[4,21]. No gross change in the location of ATP within ADK would satisfy all these criteria. *a*, The regions of the ABC cassette lacking direct counterparts in ADK (loops 1, 2, 3 and 4) are in blue. The relationship of these regions to the primary sequences are shown in Fig. 2. The N- and C-termini are indicated, as are the loops. *b*, The two Walker motifs, characteristic of many nucleotide-binding proteins, are in yellow and loop 2 in blue. *c*, The five β-sheets of the Rossmann fold[20] are in yellow. *d*, View of the structure rotated by 90°. The five β-sheets of the Rossmann fold are in pink and loop 2 is in blue.

direction. Third, the chloride channel has a rapid turnover number characteristic of channels and far greater than has been observed for any ABC transporter. Fourth, the characteristics of chloride channels differ between tissues; it is unlikely (although not impossible) that they are the products of a single gene. Finally, the molecular weight of a putative chloride carrier is much less than that of CFTR (ref. 28). The model presented here provides a basis for addressing the nature of the substrate(s) transported by CFTR, the mechanisms by which a defect in CFTR affects chloride-channel activity, as well as other mechanistic questions relating to this transport superfamily. □

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A cluster of cystic fibrosis mutations in the first nucleotide-binding fold of the cystic fibrosis conductance regulator protein

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THE gene responsible for cystic fibrosis (CF) has recently been identified and is predicted to encode a protein of 1,480 amino acids called the CF transmembrane conductance regulator (CFTR)[1,2]. Several functional regions are thought to exist in the CFTR protein, including two areas for ATP-binding, termed nucleotide-binding folds (NBFs), a regulatory (R) region that has many possible sites for phosphorylation by protein kinases A and C, and two hydrophobic regions that probably interact with cell membranes[2]. The most common CF gene mutation leads to omission of phenylalanine residue 508 in the putative first NBF, indicating that this region is functionally important[2-4]. To determine whether other mutations occur in the NBFs of CFTR, we determined the nucleotide sequences of exons 9, 10, 11 and 12 (encoding the first NBF) and exons 20, 21 and 22 (encoding most of the second NBF) from 20 Caucasian and 18 American-black CF patients. One cluster of four mutations was discovered in a 30-base-pair region of exon 11. Three of these mutations cause amino-acid substitutions at residues that are highly conserved among the CFTR protein, the multiple-drug-resistance proteins and ATP-binding membrane-associated transport proteins. The fourth mutation creates a premature termination signal. These mutations reveal a functionally important region in the CFTR protein and provide further evidence that CFTR is a member of the family of ATP-dependent transport proteins[2,5].

The association between DNA polymorphism haplotypes and specific gene mutations has been established in a variety of autosomal recessive disorders[6-9]. Therefore, we determined the haplotypes of 155 Caucasian and 43 American-black CF chromosomes using three DNA markers 5′ of the CF gene (XV2c, KM19 and Mp6d.9) and one DNA marker in the gene (G2). The three-basepair deletion mutation leading to the omission of a phenylalanine residue at codon 508 (ΔF508; F denotes Phe in the single-letter amino-acid code) was almost exclusively associated with one haplotype (E in Table 1) and accounted for 77% of the mutations on CF chromosomes from our Caucasian patients, similar to the findings of other studies of North American Caucasians[3,4]. By constrast, only 16 of 43 (37%) CF chromosomes from American-black patients had the ΔF508 mutation, confirming that racial admixture alone does not account for the incidence of CF in this group[10]. Six haplotypes are associated with 29 Caucasian CF chromosomes with unknown mutations, that is, without the ΔF508 mutation, 24 of these chromosomes having an A, D or E haplotype (Table 1). Unknown CF mutations in American-black patients are associated with a wider distribution of haplotypes than in Caucasians (Table 1).

An initial panel of 10 Caucasian CF patients having 14 of 35 unknown CF genes shown in Table 1 and representing each haplotype group was selected for nucleotide sequencing[6]. All of 18 American-black patients with 27 unknown genes were al examined. Exons 9, 10, 11, 12, 20, 21 and 22 have so far be analysed. We found four exon-11 mutations occurring in 30-base pair region; three cause amino-acid substitution whereas the fourth produces a termination codon (Fig. 1*a*). O mutation was found in 4% of Caucasian CF chromosome another occurred in 5% of American-black CF chromosome and the remaining two are rare mutations in American-bla patients (Table 2). Every mutation was present in at least o relative of each patient. To eliminate the possibility that t missense mutations are normal variants, we analysed norm chromosomes. Because neutral polymorphisms are expected occur on normal and CF chromosomes with the same haplotyp chromosomes with the same haplotype as that associated wi each mutation were selected for analysis from our group normal chromosomes (148 American black, 135 Caucasian). B this method, we examined the normal chromosomes most likel to have the sequence variation if it were a neutral polymorphis The missense mutations in exon 11 were exclusive to CF chromosomes and were never associated with normal chromosomes CF chromosomes with the ΔF508 mutation (Table 3).

The G1,778 → A (Ser549 → Asn or S549N in the single-lett amino-acid code) mutation was identified on one chromosom from an American-black CF patient and was inherited from th patient's mother. This mutation causes a conservative substitution between uncharged polar amino acids. It is associated wi haplotype A, and was not found on 40 Caucasian or 5 American-black normal chromosomes with at least two sites i common with haplotype A (Table 3).

The G1,784 → A (Gly 551 → Asp; G551D) mutation was discovered on six Caucasian chromosomes, five of which have th same 10-site haplotype D16/18. The sixth occurred on a chromosome which was identical at four sites closest to the gene (D0 haplotype in Table 3) but which differed at more distant sites So far, this is the second most common CF mutation i Caucasians. It is unlikely that this mutation is a protein polymorphism, as it replaces a neutral with a charged amino acid Furthermore, the mutation occurs on 4% of Caucasian C chromosomes in our sample and has not been found on thre normal chromosomes with the same 10-site haplotype or on 5 other normal Caucasian chromosomes. In six of seven Caucasia patients (including two siblings) who had this mutation, it wa paired with the ΔF508 mutation. Three of these patients, age 11–13 years, have mild lung disease with normal pulmonar function test results, whereas the other three patients, ages 15–1 years, have moderate-to-severe pulmonary disease. The seventh patient with the G551D mutation, age 31 years, has an unknown mutation on his other CF chromosome and has mild lung disease. All of the patients except one from the sibling pair have exocrine pancreatic insufficiency, requiring supplements of pancreatic enzymes. The range of illness severity and small number of patients precluded a meaningful assessment of the effect of this mutation on phenotype. All patients are of northern European ancestry representing different ethnic groups.

The nucleotide substitution C1,789 → T (Arg 533 → Stop; R553 Stop) is the first nonsense mutation observed in the CF gene. It occurs at a CG dinucleotide, a 'hotspot' for mutations, and it conforms to the CG → TG rule[16,17]. This mutation was found on two American-black chromosomes having haplotypes identical at eight informative sites suggesting that it has a common origin. It is not known whether a stable truncated CFTR protein is present *in vivo*; but in other disorders, nonsense mutations have been associated with unstable protein products[18]. One of the two patients with this nonsense mutation is a genetic compound with the G1,778 → A (S549N) mutation (patient 272 in Fig. 1*a*).

The fourth mutation, a G1,807 → A substitution, was found on one chromosome from an American-black patient. This mutation causes a conservative change of Ala 559 → Thr (A559T); it is associated, however, with a relatively common four-site

TABLE 1 Haplotype distributions

Haplotype	XV2c *TaqI*	KM19 *PstI*	D9 *MspI*	G2 *XbaI*	Caucasian Unk	Caucasian ΔF508	American black Unk	American black ΔF508
A	1	1	1	2	6	0	1	0
B	1	1	2	2	2	0	2	0
C	1	2	1	2	0	0	1	0
D	1	2	2	1	12	5	1	0
E	1	2	2	2	6	92	2	7
F	2	1	1	2	1	0	4	0
G	2	2	1	1	0	0	1	0
H	2	2	2	1	2	1	1	0
I	2	2	2	2	0	6	2	2
Other	–	–	–	–	6	16	12	7
					35(0.23)	120(0.77)	27(0.63)	16(0.37)

Distribution of haplotypes by race on chromosomes bearing the CF gene with an unknown (Unk) mutation or the deletion ΔF508 mutation in the Johns Hopkins database. Parentheses indicate frequency; a dash indicates polymorphism uninformative or unknown; 1 denotes the absence, and 2 denotes the presence, of a restriction site. DNA markers XV2c and KM19 and their associated polymorphisms are described for these populations elsewhere[10]. Probes D9 (Mp6d.9) and G2, which detect *MspI* and *XbaI* polymorphic sites respectively, were obtained from Professor R. Williamson[11,12]. Direct detection of the ΔF508 mutation was performed by polymerase chain reaction (PCR) amplification of genomic DNA using primers C16B and C16D followed by vacuum blotting of amplified DNA to nitrocellulose filters and hybridization with radioactively labelled oligonucleotides of either normal sequence or deletion F508 sequence as previously described[3].

TABLE 2 Mutations detected in exon 11 of the CF gene

Mutation: Nucleotide	Mutation: Amino acid	CF chromosome: Racial origin	CF chromosome: Haplotypes (no. of chromosomes)*	Restriction-enzyme site change caused by mutation
G1,778 → A	Ser 549 → Asn	American black	A15 (1)	Loss of *DdeI* site†
G1,784 → A	Gly 551 → Asp	Caucasian	D16/18 (5) D03 (1)	Loss of *HincII* site‡ Creation of *MboI* site§
C1,789 → T	Arg 553 → Stop	American black	I12 (1) Ii06 (1)	Loss of *HincII* site‡
G1,807 → A	Ala 559 → Thr	American black	F (1)	None

* Haplotype codes: first capitalized letter, four-site (XV2c, KM19, D9 and G2) haplotype shown in Table 1; numbers following the letter, extended haplotype (7C22, MET, D7S8); i, incomplete haplotype informative for at least three of the sites in the four-site haplotype.

† PCR amplification of genomic DNA using exon-11 primers 11i-5′ and 11i-3′ (see Fig. 1*a*) produces a 425-base-pair (bp) fragment. *DdeI* digestion of DNA amplified from normal exon-11 sequence creates two fragments of 174 and 251 bp, whereas DNA amplified from exon 11 sequence containing this mutation will not be cut with *DdeI*.

‡ *HincII* digestion of DNA amplified from normal exon-11 sequence (425 bp) produces fragments of 186 and 239 bp. The Gly 551 → Asp and Arg 553 → Stop mutations both remove the normal *HincII* recognition site.

§ DNA amplified from normal exon-11 sequence (425 bp) cannot be cut with *MboI*, whereas digestion of DNA amplified from exon-11 sequence containing this mutation will create two fragments of 182 and 243 bp.

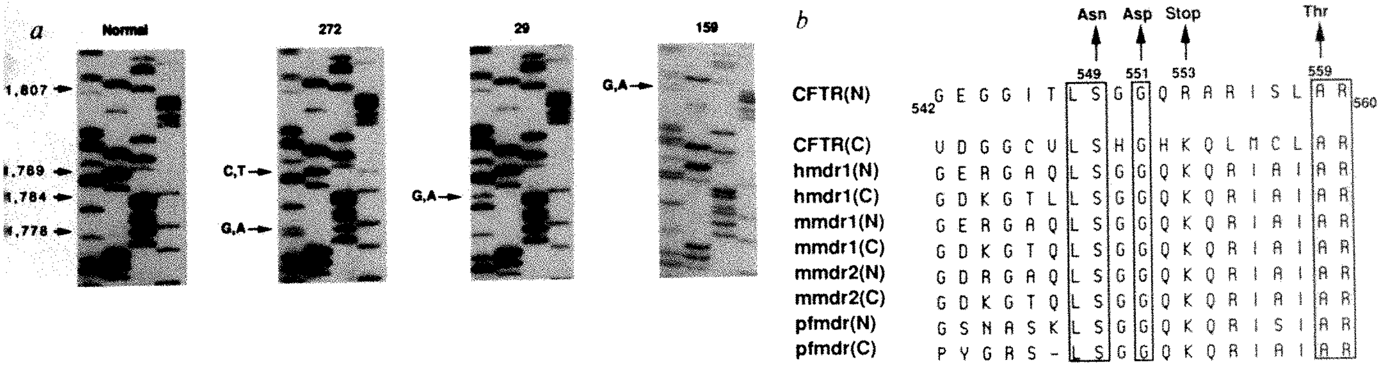
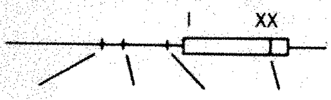

a
Normal
272
29
159
1,807
1,789
1,784
1,778
C,T
G,A
G,A
G,A
b
Asn
Asp
Stop
Thr
549
551
553
559
542
560
CFTR(N) G E G G I T L S G G Q R R R I S L R R
CFTR(C) U D G G C U L S H G H K Q L M C L R R
hmdr1(N) G E R G R Q L S G G Q K Q R I R I R R
hmdr1(C) G D K G T L L S G G Q K Q R I R I R R
mmdr1(N) G E R G R Q L S G G Q K Q R I R I R R
mmdr1(C) G D K G T Q L S G G Q K Q R I R I R R
mmdr2(N) G D R G R Q L S G G Q K Q R I R I R R
mmdr2(C) G D K G T Q L S G G Q K Q R I R I R R
pfmdr(N) G S N R S K L S G G Q K Q R I S I R R
pfmdr(C) P Y G R S - L S G G Q K Q R I R I R R

FIG. 1 *a*, Direct sequence analysis of PCR-amplified genomic DNA from exon 11 of CFTR using the primer 11i-5′. The order of nucleotides is A, C, G, T. Normal sequence extending from nucleotide position 1,769 to 1,816 is shown beside sequence obtained from patients 272, 29 and 159. Patient 272 has exon 11 mutations on each chromosome, G1,778 → A (G,A) and C1,789 → T (C,T); patient 29 has the mutation G1,784 → A; patient 159 has the mutation G1,807 → A. *b*, Alignment of predicted amino-acid sequence (single-letter code) from residues 542–560 in exon 11 of the first NBF with conserved regions in the second NBF of CFTR and similar regions in four members of the multiple drug resistance (mdr) protein family. Positions of the amino-acid substitutions and stop signal found in this study are shown. Amino acids conserved in all 10 sequences are boxed. Adapted from ref. 2. N, N terminal; C, C terminal; h, human; m, mouse 1 and 2; pf, *Plasmodium falciparum* mdr proteins.

METHODS. PCR amplification was performed as previously described[13] using oligonucleotide primers 11i-5′ (5′-CAACTGTGGTTAAAGCAATAGTGT-3′) and 11i-3′ (5′-GCACAGATTCTGAGTAACCATAAT-3′) selected from intron sequences flanking 5′ and 3′ of exon 11 of the CFTR gene. About 500 ng genomic DNA from peripheral lymphocytes of each subject was amplified using 2 μl of a 10 μm solution of each primer described above in a total volume of 100 μl containing *Taq* polymerase buffer (50 mM KCl, 10 mM Tris buffer, pH 8.3, 1.5 mM $MgCl_2$, 0.01% (w/v) gelatin), 0.02 μm each 2′-deoxynucleotide 5′-triphosphate (Pharmacia) and 2.5 U *Taq* polymerase (Cetus). Amplification was performed by 30 cycles of annealing at 58 °C for 30 s, extension at 72 °C for 1 min and denaturing at 94 °C for 30 s. Sequencing of DNA amplified from genomic DNA was performed as described[14,15].

TABLE 3 DNA polymorphism haplotypes associated with the exon-11 missense mutations



Haplotype*	XV2c *T*	KM19 *P*	D9 *M*	G2 *X*	CF chromosomes†			Normal chromosomes‡		
Mutation G1,778 → A					G1,778 → A	Normal at 1,778 Caucasian	Normal at 1,778 Black	G1,778 → A	Normal at 1,778 Caucasian	Normal at 1,778 Black
A15	1	1	1	2	1	0	0	0	0	0
A	1	1	1	2	0	5§	0	0	32	6
1c	—	1	1	2	0	1	4	0	0	29
c	—	—	1	2	0	0	3	0	8	18
Other	—	—	—	—	0	27	19	0	40	40
					1	33	26	0	80	93
Mutation G1,784 → A					G1,784 → A	Normal at 1,784 Caucasian	Normal at 1,784 Black	G1,784 → A	Normal at 1,784 Caucasian	Normal at 1,784 Black
D16/18	1	2	2	1	5	0	0	0	3	0
D03	1	2	2	1	1	5	0	0	1	0
D	1	2	2	1	0	1	1	0	7	1
Other	—	—	—	—	0	21	26	0	46	35
					6	27	27	0	57	36
Mutation G1,807 → A					G1,807 → A	Normal at 1,807 Caucasian	Normal at 1,807 Black	G1,807 → A	Normal at 1,807 Caucasian	Normal at 1,807 Black
F	2	1	1	2	1	1	3	0	ND	8
1c	—	1	1	2	0	7	4	0	ND	17
c	—	—	1	2	0	0	3	0	ND	2
Other	—	—	—	—	0	22	16	0	ND	9
					1	30	26	0		36

Map at the top shows the relative positions and approximate distances between the markers XV2c, KM19, D9 and G2 relative to the 250-kilobase CF gene, which is shown as a box[3,11,12]. Roman numerals denote exons 1 and 20 respectively.

* Extended haplotypes include 7C22, MET and D7S8 sites which are not shown (see Table 2); 1 indicates the absence and 2 the presence of a particular site;-, site is different or uninformative. Enzyme abbreviations: *T*, *Taq*I; *P*, *Pst*I; *M*, *Msp*I; *X*, *Xba*I.

† CF chromosomes shown do not have the ΔF508 mutation. Fifty-six Caucasian and nine American-black chromosomes with the ΔF508 mutation did not have the G1,778 → A or G1,784 → A mutations and twenty Caucasian and nine American-black chromosomes with the ΔF508 mutation did not have the G1,807 → T mutation. Fifty Caucasian CF and twenty-six black CF chromosomes were directly sequenced in each case.

‡ Normal Caucasian chromosomes are from parents and/or siblings of CF patients and are therefore non-CF-gene bearing. Normal black chromosomes are either non-CF-gene-bearing chromosomes from healthy family members or chromosomes from black patients heterozygous for sickle-cell anaemia or β-thalassemia (CF carrier frequency in American blacks is 1 in 65 persons[10]). Normal chromosomes with four-site haplotypes (XV2c, KM19, Mp6d.9 and G2) identical to the mutation-bearing chromosomes were examined whenever possible. But two- or three-site haplotypes, which included the intragenic marker G2 and the closest 5′ markers (Mp6d.9 ± Km19), were also employed. Screening of normal chromosomes and additional Caucasian CF chromosomes for each mutation was as follows: PCR amplification of exon 11 followed by *Dde*I digestion to detect the G1,778 → A mutation or *Mbo*I digestion to detect the G1,784 → A mutation or direct sequencing to detect the G1,807 → A mutation (Table 2). ND, not determined.

§ DNA from only five of the six Caucasian patients with an unknown mutation associated with haplotype A was available.

haplotype (F) in the American-black population. Direct sequencing of eight normal chromosomes with the same four-site haplotype, 19 other normal chromosomes with at least two sites in common with haplotype F, and nine additional normal chromosomes from American-black sickle-cell or β-thalassemia carriers did not reveal this mutation.

The CF gene was identified solely by its location in the human genome[1]. Little is known of the function of its protein product except by analogy with well-characterized proteins that have similar amino-acid sequences[2]. Nucleotide sequencing of six other exons of the CF gene (exons 9, 10, 12, 20, 21 and 22) has identified only isolated mutations (G. C., unpublished results). The four mutations described here occur in a 13-amino acid segment (codons 548-560) of the putative first NBF in the CFTR protein, indicating that this region is functionally important. This 13-amino acid segment is highly conserved with similar regions of other membrane-associated transport proteins[2]. Five amino acids in this region are completely conserved in comparable regions from the multiple-drug-resistance proteins, indicating that these positions are probably crucial to protein function (Fig. 1*b*). The amino-acid substitutions described in this study occur at three of the five completely conserved residues. Moreover, the substitutions occur at the three most conserved residues in this region among CFTR and 14 other membrane-associated proteins that bind ATP (see in ref. 2). Therefore, the location of this cluster of mutations supports the theory that the CFTR protein is a member of the superfamily of ATP-dependent transport proteins[2,5]. ☐

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No evidence for expression of the insulin-regulatable glucose transporter in endothelial cells

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A MAJOR effect of insulin is to increase glucose transport in muscle and fat[1,2]. A family of genes encoding distinct mammalian glucose transporters has recently been elucidated[3–9]. One of these, the insulin-regulatable glucose transporter (IRGT)[3,10], is primarily expressed in muscle and fat, tissues that exhibit insulin-dependent glucose transport. Insulin promotes glucose transport in these tissues by stimulating movement of the glucose transporter from an intracellular location to the plasma membrane[3,10–14]. Recent studies[15], however, suggest that an additional effect of insulin in these tissues may be the facilitation of glucose transport, presumably across capillary endothelium. This hypothesis is based on the localization of the IRGT in endothelial cells specific to muscle and adipose tissue. We report here, however, on morphological and biochemical studies using several different IRGT-specific antibodies in which we could not reproduce these results.

Immunohistochemical analyses were performed using a monoclonal (1F8) and two polyclonal (anti-IRGT$_{12}$ and anti-IRGT$_{19}$) antibodies against the IRGT. The monoclonal antibody was produced by immunization of mice with intracellular vesicles from rat adipocytes[10]. Anti-IRGT$_{12}$ and anti-IRGT$_{19}$ are specific for synthetic peptides encompassing the last 12 and 19 amino acids respectively, of the IRGT C terminus. Light microscopy of cardiac muscle from non-stimulated rats, immunolabelled with anti-IRGT antibodies (Fig. 1*a,b*) indicated pronounced labelling of intracellular structures close to the nuclei (n) and near the lateral cell surface of myocytes (arrowheads). These are areas where the regular myofibril pattern is disturbed by accumulated cell organelles, mostly mitochondria. In addition, some labelling was associated with the Z-lines in cardiac myocytes. In skeletal muscle (Fig. 1*c,d*), labelling of the IRGT was confined to regions at the cell borders of the myocytes. No clear labelling of the Z-lines was observed in these cells. Changes in the labelling pattern in either cardiac or skeletal muscle following insulin stimulation were not as obvious as in renal brown adipose tissue. In brown adipocytes from insulin-treated rats, IRGT labelling was evident along the entire cell surface (Fig. 1*e,f*), including the domains facing the blood capillaries (c) and the borders between the adipocytes (arrowheads). Most of the adipocyte surface-labelling could be assigned to the plasma membrane by electron microscopy (Fig. 2). Some labelling was also associated with intracellular structures (Fig. 1*e*, double arrows). In brown adipocytes from non-insulin treated rats, the cell surface was devoid of IRGT-labelling and the transporter was localized primarily to an intracellular domain (results not shown). These observations are compatible with the current view that the IRGT moves from an intracellular

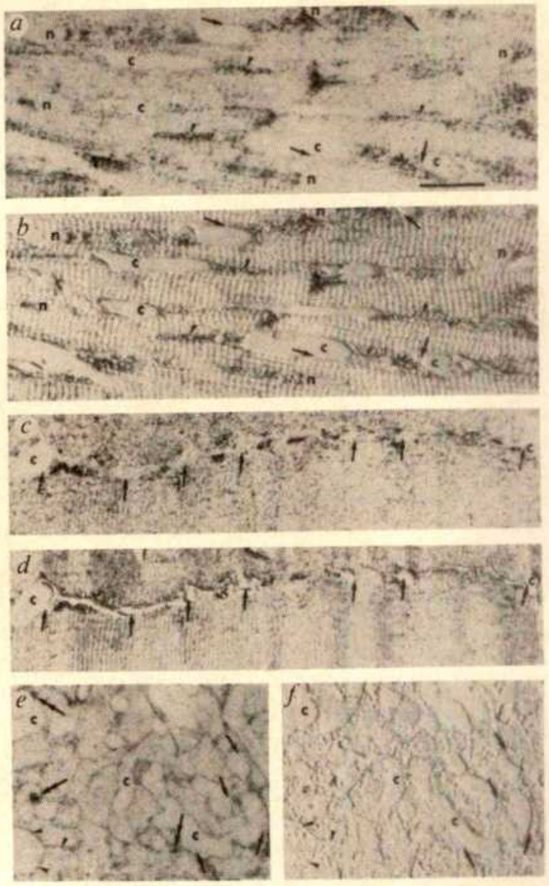

FIG. 1 Distribution of the IRGT in muscle and adipose tissue. Shown here are light micrographs of gold labelling in cryosections visualized by silver enhancement (*a*, *c* and *e*) and the Nomarski image to provide the corresponding structural information (*b*, *d* and *f*). *a*, *b*, Cardiac muscle, non-stimulated, labelled with anti-IRGT$_{19}$; *c*, *d*, skeletal muscle, non-stimulated, labelled with anti-IRGT$_{12}$; *e*, *f*, brown adipose tissue, insulin-stimulated, labelled with monoclonal antibody 1F8. Blood capillaries are marked by C. Further marking is explained in the text. Magnification is similar for *a*–*f*. Bar in *a*, 20 μm.

METHODS. Cardiac and skeletal (soleus) muscle were obtained from ~100 g Sprague Dawley rats following perfusion fixation with 0.1 M phosphate buffer (pH 7.4) containing 2% paraformaldehyde and 0.2% glutaraldehyde. Insulin-stimulated rats were fasted overnight and injected with glucose and insulin (1 g glucose, 8 U insulin in 3 ml saline per kg body weight, i.p.), 30 min before fixation. Nonstimulated rats received a saline injection (3 ml saline per kg, i.p.) instead. Renal brown adipose tissue was prepared under identical conditions, except that rats were kept at 4 °C (4 h) before fixation in order to reduce intracellular triglyceride levels. Comparative studies performed in animals not incubated at 4 °C indicated that this treatment did not affect the distribution of the IRGT (data not shown). Tissues were kept for 2 h at 20 °C in fixative before the preparation of semi-thin cryosections (~300 nm). Sections were incubated with antibodies as previously described. Three different anti-IRGT antibodies were used for immunolabelling: two rabbit polyclonal antibodies against either a 12-amino-acid peptide[3] (anti-IRGT$_{12}$), or a 19-amino-acid-peptide (anti-IRGT$_{19}$) encompassing the C terminus of the IRGT sequence, or a monoclonal antibody (1F8) raised against the rat adipocyte IRGT[10]. The anti-IRGT$_{12}$ and 1F8 were affinity-purified using a protein A Sepharose affinity matrix (BioRad) and used for labelling at concentrations of 5 and 2.5 μg ml^{-1} respectively. The anti-IRGT$_{19}$ was affinity-purified using the peptide antigen conjugated to acrylamide beads (BioRad) and used for labelling at a concentration of 1–5 μg ml^{-1}. Labelling with the polyclonal antibodies was detected by light microscopy following incubation with swine anti-rabbit IgG, and protein A/gold as previously described[19], and immunolabelling with monoclonal antibody 1F8 was visualized following incubation with rabbit anti-mouse IgG and protein A gold. In control sections (not shown), incubations were performed with either a non-specific antibody, or with anti-IRGT$_{12}$ and anti-IRGT$_{19}$ mixed 1 h before use with a 10-fold molar excess of the 12- or 19-amino-acid peptide antigen, respectively. In each case the labelling pattern observed in IRGT-labelled sections was completely absent in the controls.

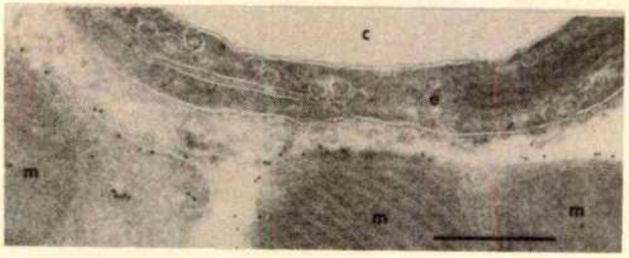

FIG. 2 Electron micrograph of a thin brown adipose tissue cryosection from an insulin-treated rat, immunolabelled with anti-$IRGT_{19}$ followed protein A gold (10 nm markers). Fragments of adipocytes with gold labelli along the plasma membrane are shown below. m, Mitochondria. endothelial cell (e) that lines the capillary space (c) is not significan labelled. In 5 sections from 5 different insulin-treated rats, we located low magnification 5–10 cross-sectioned capillaries. Quantitation of gold within 20 nm from the plasma membrane of endothelial cells and th surrounding plasma membrane of the adipocytes, indicated that the labelli density over luminal and adluminal membranes was 5.2 ± 2.4% (s.d.) an 6.5 ± 2.7%, respectively, of the gold labelling density in association with th corresponding adipocyte membrane. Bar, 0.5 μm.

region to the cell surface following insulin administration *in vivo*[3,10–12].

At present we are performing an extensive electron microscopic analysis to characterize the distribution of the IRGT in brown adipose tissue and muscle from animals that have been treated with saline or insulin. Preliminary results have shown that the main intracellular location of the IRGT in adipocytes, as well as in myocytes, is in characteristic small tubular and vesicular structures. As in the cardiac muscle cell (Fig. 3, *a–c*), many of these labelled structures occur in large agglomerations that are associated with the Golgi complex, representing the trans Golgi reticulum (TGR in Fig. 3*a*). In addition, many of the labelled vesicles and tubules are scattered throughout the cytoplasm, often in close proximity to the cell surface (Fig. 3*b*) and, only in the cardiac myocyte, at the Z-line level often near the transverse (T-) tubules (Fig. 3*c*).

The reactions observed were essentially the same for the three anti-IGRT antibodies, except that 1F8 occasionally gave some spurious reactions (see below). No reaction was observed in control sections incubated with nonspecific antibodies or with anti-IRGT polyclonal antibodies, when preincubated with the appropriate peptides. The reactivity of 1F8 was quenched afte incubating that antibody with one of the peptides, indicatin that the epitope of the monoclonal may be at the C-termina tail of the IRGT as well.

We have observed labelling of the IRGT only in adipocyte and myocytes, but not in other cell types such as endothelia cells (Figs 1, *a–f*, arrows; 2 and 3*b*). Quantitation of gol particles in IRGT-labelled brown adipose tissue sections o insulin-treated animals indicated that the labelling density a the surface of endothelial cells was only 5–7% of that associate with the cell surface of adipocytes (Fig. 2). These data are i direct contrast to a recent study using monoclonal antibody 1F (ref. 15) in which intense labelling of the endothelial cells specifi to muscle and fat tissue and only poor labelling of the corresponding myocytes or adipocytes were reported. To clarify thi anomaly, we have isolated endothelial cells from rat epididyma fat pads and have been unable to detect the IRGT in thes isolated cells by biochemical means, using the anti-IRGT antibodies employed in this study. In contrast, thrombomodulin, an endothelial cell specific marker[16], was highly enriched in ou endothelial cell fraction but undetectable in the adipocyte frac-

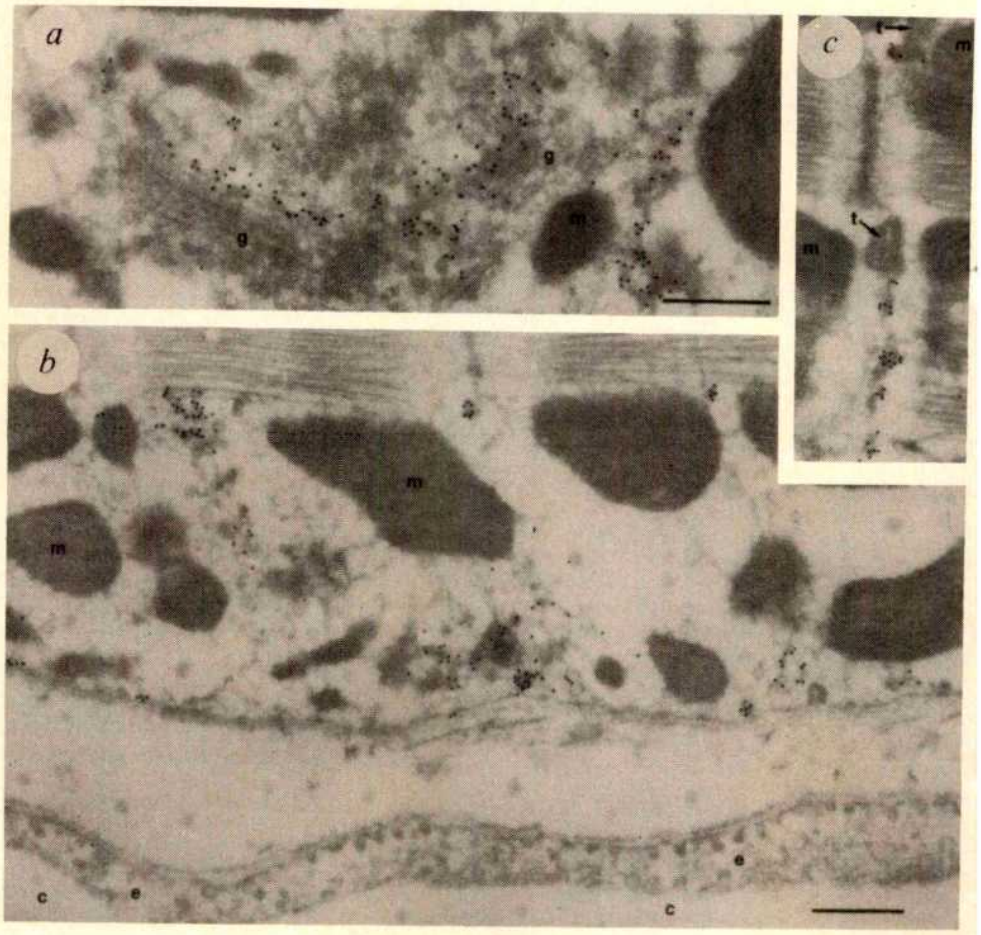

FIG. 3 Electron micrographs showing the IRGT distribution in cardiac muscle from a nonstimulated rat. The section was prepared as for Fig. 2, except that anti-$IRGT_{19}$ incubation was followed by swine anti-rabbit IgG and then protein A/gold (15 nm marker). *a*, IRGT is abundant in a tubulo-vesicular system, presumably the TGR, near the Golgi complex (g). *b*, Similar labelled elements occur in small groups or isolated, near the cell surface; *c*, localization in the cytoplasm between the myofibirils, most frequently at the Z-line level of the sarcomeres close to the surface connected T-tubules (t). No noticeable labelling is present in the endothelial cell (e). Capillary space, c. Bar, 0.5 μm; magnification in *c* as in *b*.

ion. All immunoreactive IRGT present in adipose tissue was etected in the isolated adipocyte fraction (Fig. 4).

We conclude that the level of expression of the IRGT in ndothelial cells is insignificant compared with that observed n myocytes and adipocytes. Using three different antibodies we ave consistently observed negligible labelling of endothelial ells in skeletal muscle, heart and adipose tissue of five nonnsulin-treated and five insulin-treated rats, except for an ccasional, spurious reaction with 1F8. We observed other faulty eactions with 1F8, for instance in nuclei of all cell types and n the lumen of myocyte sarcoplasmic reticulum. These observaions are at odds with the apparent cytosolic orientation of the epitope for 1F8. These spurious reactions, including those bserved in endothelial cells, were not consistently present in 1F8-labelled tissue sections and never in the immunoblotting experiments. Moreover, this aberrant labelling pattern was never observed using polyclonal antibodies. We were able to compete out both 1F8 and the polyclonal antibody immunolabelling of cells when the antibodies were preincubated with a 10-fold molar excess of the IRGT C-terminal peptide. In fact this was even evident with respect to the endothelial cell and nuclear staining of 1F8. This might indicate that the spurious labelling observed with 1F8 is due to weak cross reaction with other than IRGT molecule(s). Most probably this can only result in low stringent binding, so that minor variations in the reaction conditions can explain why the cross reaction is only sometimes manifest in immunolabelled preparations, and why these spurious 1F8 reactions are not reported from immunoprecipitation[10] or immunoblotting (Fig. 4) studies. These observations suggest that localization of the IRGT using only 1F8 may be difficult to interpret and we suspect that the previous data[15] may largely reflect the aberrant labelling pattern of the monoclonal antibody.

Our data indicate that it is the permeability of the myocyte or adipocyte membrane that limits entry of glucose into these cells. Previous studies have shown quite convincingly that in cardiac muscle there is no significant difference in transcapillary flux of L- compared with D-glucose[17,18]. Thus, the existence of a transporter to facilitate the movement of glucose out of the endothelium, at least in cardiac muscle, would seem to be redundant. We conclude that, in muscle and adipose tissue, the IRGT is primarily expressed in myocytes and adipocytes, respectively. In these cells the transporter is enriched in the trans Golgi reticulum and in small vesicles and tubules scattered throughout the cytoplasm. These sites of IRGT seem to be in an insulin-regulated equilibrium with the plasma membrane. □

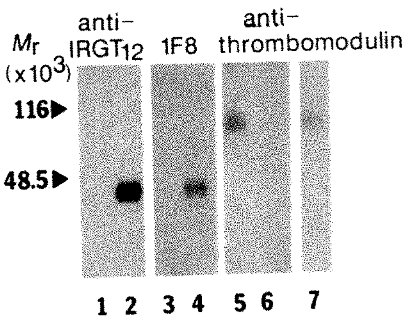

FIG. 4 Distribution of the IRGT (lanes 1-4) and thrombomodulin (lanes 5-7) in endothelial cells (lanes 1, 3 and 5) and adipocytes (lanes 2, 4 and 6) isolated from rat epididymal fat pads. Aliquots (10 μg total protein) of the homogenates were immunoblotted with either anti-IRGT$_{12}$ (lanes 1, 2), 1F8 (lanes 3, 4) or anti-thrombomodulin[21] (lanes 5, 6, 7). An aliquot of purified rat thrombomodulin was also immunoblotted as a reference (lane 7). Relative molecular weights (M_r) are indicated on the left.

METHODS. Epididymal fat pads were dissected from male rats (150 g) and digested with collagenase for 45 min at 37 °C as previously described[11,12]. Endothelial cells were prepared essentially as described[20]. Following collagenase digestion, cells were passed through a 250-μm pore nylon filter. The fat cells were allowed to float to the surface and removed. The remaining filtrate was centrifuged at 400*g* (5 min). The pellet was washed with Krebs Ringer buffer and passed through a 20-μm pore nylon filter. The clumps of endothelium that remained attached to the filter were washed in buffer and recentrifuged. Inspection of this fraction by light microscopy revealed an identical morphology to that reported previously for microvascular endothelium with little contamination from cells of other origin[20]. From 50 rats we isolated ~250 μg of endothelial cell protein.

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Suppression of experimental glomerulonephritis by antiserum against transforming growth factor β1

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GLOMERULONEPHRITIS is an inflammation of the kidney characterized by the accumulation of extracellular matrix within the damaged glomeruli[1-4], impaired filtration and proteinuria. In its progressive form, the disease destroys kidney function leading to uraemia and death, unless dialysis therapy or kidney transplantation is available. The pathogenesis of glomerulonephritis is incompletely understood, but the eliciting factor is thought often to be an immunological injury to mesangial and/or other resident cells in the glomeruli[5,6]. We have used an animal model of acute mesangial proliferative glomerulonephritis[7,8] to show that this disease is associated with increased production and activity of transforming growth factor β1 (TGF-β1)[9], an inducer of extracellular matrix production[10-17]. Here we report that administration of anti-TGF-β1 at the time of induction of the glomerular disease suppresses the increased production of extracellular matrix and dramatically attenuates histological manifestations of the disease. These results provide direct evidence for a causal role of TGF-β1 in the pathogenesis of the experimental disease and suggest a new approach to the therapy of glomerulonephritis.

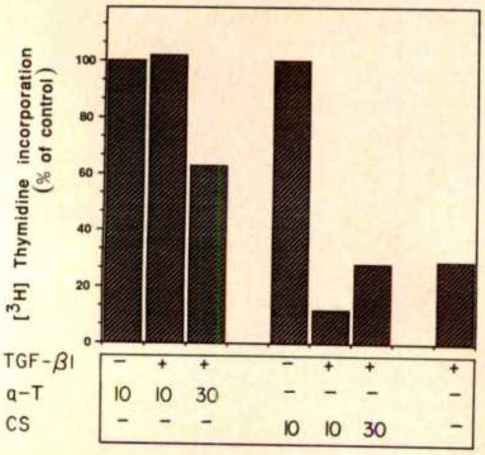

FIG. 1 Inhibition of TGF-β1 activity by antibodies. A mink lung epithelial ce assay that measures growth inhibition by TGF-β (ref. 30) was used to ass the ability of anti-TGF-β antiserum to neutralize its activity. Dilutions 1:10 (10) and 1:30 (30) of anti-TGF-β1 serum (a-T) inhibited the action TGF-β1 (TGF-β1) added to the cultured cells. Control antiserum (CS) had r effect (+, added; −, not added).

METHODS. Anti-TGF-β1 serum was prepared against a synthetic peptic from residues 78–109 (ref. 18) of human mature TGF-β1. The peptide wa synthesized in an Applied Biosystems solid phase peptide synthesizer ar purified by HPLC. The rabbit was immunized subcutaneously with 2 mg pe injection of the peptide which was mixed with 0.5 mg methylated BSA and emulsified in Freund's complete adjuvant. The injections were generall given 4 weeks apart and the rabbit was bled about 1 week after the secon and every successive injection. The antisera used in this work had a titr (50% binding) of 1:6,000 in radioimmunoassay, bound to TGF-β1 i immunoblots and inhibited the induction of proteoglycan synthesis cause by TGF-β1 in cultured mesangial cells (results not shown). Growth inhibitio was assayed by adding to mink lung epithelial cell cultures 3 ng ml^{-1} huma platelet TGF-β1 (Calbiochem) which had been preincubated with dilutions c 1:10 or 1:30 of heat-inactivated (56 °C, 30 min) anti-TGF-β1 serum or wit identical dilutions of a control antiserum prepared against an unrelate peptide (from the cytoplasmic domain of the α_4-subunit of an integrin followed by an assay for [^{3}H]thymidine incorporation[30].

Glomerular mesangial cells express a Thy-1.1 epitope on their surface[7]. Injection of antithymocyte serum into an animal produces a dose- and complement-dependent selective injury to mesangial cells resulting in acute mesangial proliferative glomerulonephritis[7,8]. The glomerular lesion is characterized by expansion of the mesangial matrix and hypercellularity, resembling the morphological features of human mesangial proliferative glomerulonephritis.

Induction of glomerulonephritis in rats with a single injection of antithymocyte serum was followed by treatment with an injection of either anti-TGF-β1 or control sera. The anti-TGF-β1 serum was prepared by immunizing a rabbit with a synthetic peptide containing residues 78–109 from human mature TGF-β1; the cyclized form of this peptide elicits an antiserum capable of inhibiting binding of TGF-β1 to cells[18]. The specificity of the antiserum was established as described in the legend of Fig. and it can neutralize the activity of purified TGF-β1 in a bioassa (Fig. 1).

The effects of the anti-TGF-β antiserum and control sera o the glomerulonephritis model were evaluated by testing 1 animals in each group in three different experiments. We use histological evaluation of glomerular extracellular matri accumulation as a measure of disease activity, because it is characteristic feature of acute glomerulonephritis[1-4].

Figure 2 shows a comparison of the microscopic appearanc of representative glomeruli, and measurement of the glomerula extracellular matrix is shown in Fig. 3. The glomeruli contai much less extracellular matrix material in the anti-TGF-β1- treated groups than in the groups receiving control serum Immunofluorescent staining of the tissues with antibodie

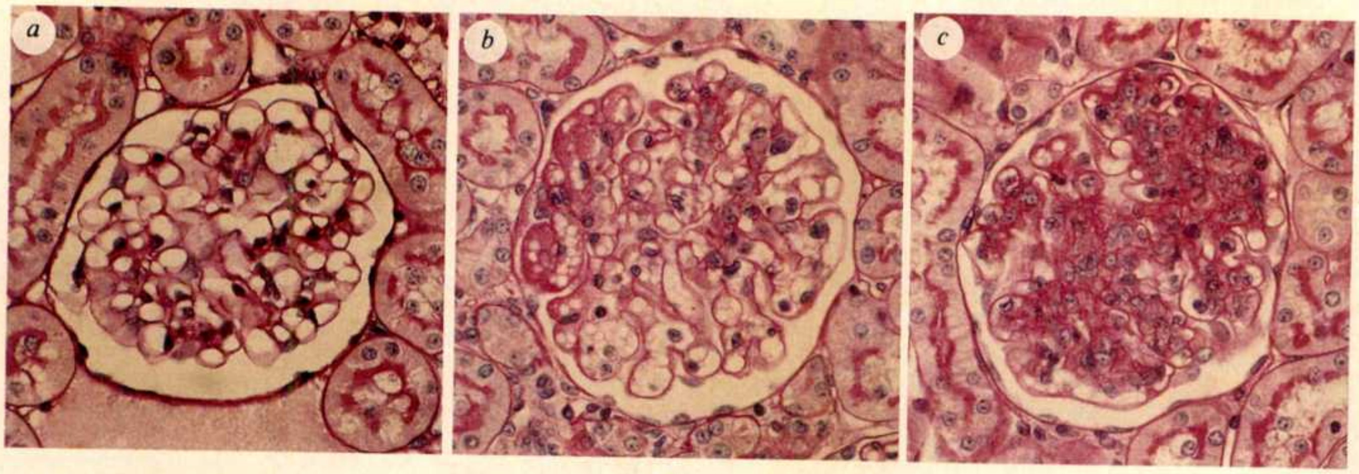

FIG. 2 Histological analysis showing pathological changes in glomeruli of glomerulonephritic kidneys. Micrographs showing periodic acid-Schiff staining of glomeruli. *a*, Staining of the basement membrane and extracellular matrix in a normal glomerulus. *b*, From a rat on day 7 after the injection of antithymocyte serum. This rat received injections of 1 ml rabbit anti-TGF-β1 on six successive days, starting on the day of the antithymocyte injection. *c*, From an animal that received control serum under a similar regimen. A striking increase in extracellular matrix is seen as the reddish-pink amorphous material filling most of the glomerulus in *c*; *b* shows a clear effect of anti-TGF-β1 in preventing the increase in glomerular extracellular matrix that occurs on day 7 after injection of antithymocyte serum. Magnification, ×500.

METHODS. Antithymocyte serum was produced by immunizing New Zealand white rabbits with 1×10^8 rat thymocytes in complete Freund's adjuvant, followed by boosting with 1×10^8 thymocytes given intravenously 2 and 4 weeks later[9]. The serum was absorbed three times each with packed rat erythrocytes and rat liver powder to remove nonspecific reactivity. Glomerulonephritis was induced in Sprague Dawley rats (4–6 weeks old) by intravenous (i.v.) administration of 1 ml antithymocyte serum per 100 g body weight[9]. The anti-TGF-β1 serum and the two rabbit sera used as controls were also administered i.v. All sera were heat-inactivated at 56 °C for 30 min before injection. The extent of glomerular injury was evaluated by performing glomerular cell counts from 30 randomly selected glomeruli from 10 normal animals and nephritic animals in each group on days 4 and 7. Normal rat glomeruli contained 52 ± 14 cells. On day 4 there was a decreased number of cells (35 ± 11) as a result of cell lysis by the antithymocyte antibody, whereas an increased number of cells was seen on day 7 (68 ± 15). Values are mean ± s.d. The changes in cellularity were the same in the anti-TGF-β1 treatment and control groups.

against fibronectin, laminin and type IV collagen showed that the pathological matrix, as well as the normal matrix, contained each of these proteins (results not shown).

In a previous study we found that the injured glomeruli in culture produce more extracellular matrix components than do cells from normal glomeruli, and that the synthesis of two proteoglycans, biglycan and decorin, is particularly high[9]. This pattern of proteoglycan synthesis is similar to that obtained by adding TGF-β1 to cultures of rat mesangial cells from normal glomeruli[17], and antibody inhibition experiments and TGF-β assays have shown that it is caused by increased synthesis of TGF-β1 in the glomeruli[9]. We therefore used the production of proteoglycans as a bioassay that would reflect the activity of TGF-β and its influence on extracellular matrix synthesis in our disease model. Such an analysis showed that proteoglycan production by glomerular cells was suppressed to a near normal rate by anti-TGF-β1 (Fig. 4*a*). Scans of the gel bands (Fig. 4*a*) and other similar experiments indicated that the suppression of this measure of the disease process was about 60% on day 4 and 80% on day 7 after injection. These results show that the glomerular disease was substantially attenuated by the anti-TGF-β1 treatment.

The level of TGF-β1 messenger RNA in the glomeruli of glomerulonephritis rats is higher than in normal rats and these glomeruli contain more cells producing TGF-β1 than do normal glomeruli[9]. These results indicate that increased expression of the TGF-β1 gene is correlated with injury in this model. Messenger RNA analysis of the anti-TGF-β1-treated and control glomeruli on the current study revealed equally raised levels of TGF-β1 mRNA in both groups of rats (Fig. 4*b*). A similar increase in the number of TGF-β1-positive cells was also seen in the treated and control animals (results not shown). These results indicate that anti-TGF-β1 did not interfere with induction of glomerular injury, as reflected by the increased synthesis of TGF-β1 mRNA and TGF-β1 protein in glomeruli. The cells responsible for the increased TGF-β1 expression have not been identified, but they could be proliferating mesangial cells and/or infiltrating monocyte/macrophages.

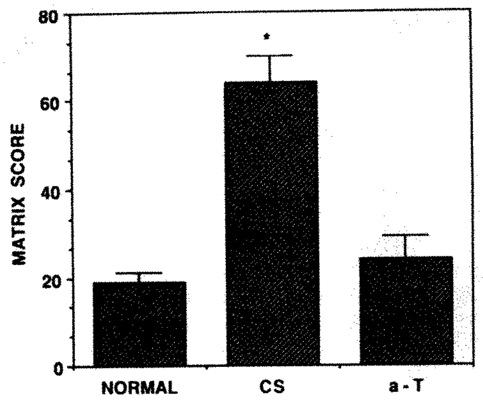

FIG. 3 Quantitation of extracellular matrix accumulation in nephritic glomeruli. Asterisk denotes $P < 0.001$, for glomerulonephritic rats treated with control serum (CS) compared with anti-TGF-β1 (a-T) on day 7 of glomerulonephritis. Normal (normal) rats were included for comparison. Values are mean ±s.d. METHODS. To quantitate mesangial matrix all sections were coded and read by an observer unaware of the experimental protocol applied. Thirty glomeruli (80–100 μm in diameter) were selected at random in sections prepared from normal rats or from anti-TGF-β1 rats and control-treated rats on day 7 of glomerulonephritis. The degree of glomerular matrix expansion was determined as the percentage of each glomerulus occupied by mesangial matrix by using a published method[31]. Differences between groups in matrix scores were analysed by *t*-test. Two types of control sera were used: a normal rabbit serum, and a rabbit antiserum prepared against an unrelated peptide. Neither had any effect on the glomerulonephritis, and the results were pooled for the figure.

Our findings establish a central role for TGF-β in the pathogenesis of acute mesangial proliferative glomerulonephritis. TGF-β can greatly stimulate the production of extracellular matrix components by various kinds of cells[10-16], including the production of the two proteoglycans[20,21] we have used as markers of TGF-β activity in this and earlier studies[9,17]. Because of this activity, TGF-β has been suspected to have a role in fibrotic diseases resulting from various chronic disease processes[22,23]. Whereas earlier results have provided correlative evidence for a role of TGF-β in glomerulonephritis, the antibody-inhibition data presented here establish that TGF-β has a direct, causal role, at least in the experimental glomerulonephritis model we have used. The type of TGF-β responsible for the increased production of extracellular matrix in our model

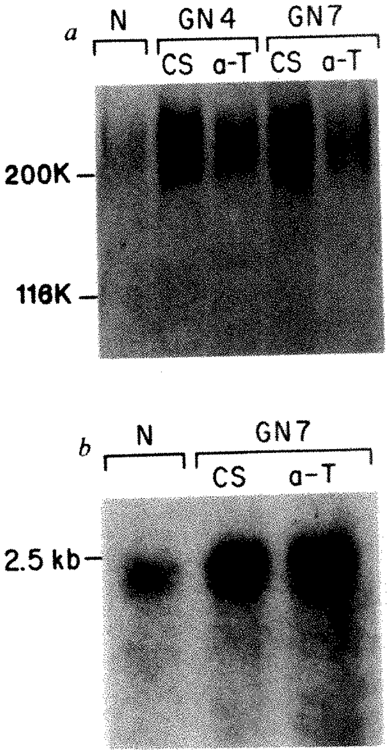

FIG. 4 *a*, Proteoglycan synthesis by glomeruli from glomerulonephritic rats treated with anti-TGF-β1. CS, glomerulonephritic rats treated with control serum; a-T, glomerulonephritic rats treated with rabbit anti-TGF-β1 on days 4 (GN 4) and 7 (GN 7) after injection of antithymocyte serum. The control lane (N) shows proteoglycan production in glomeruli from normal rat kidney. *b*, Northern blotting of TGF-β1 mRNA in glomeruli isolated from the kidneys of normal and treated glomerulonephritic rats. Scanning of the bands showed a fivefold increase, relative to normal controls, in TGF-β1 mRNA in both anti-TGF-β1 (a-T) treatment and control (CS) groups on day 7. The control lane (N) shows TGF-β1 mRNA in glomeruli from normal rat kidney.
METHODS. *a*, Glomeruli were isolated 4 and 7 days after an antithymocyte serum injection that had been followed by treatments similar to those described in the legend of Fig. 3, and placed in culture[9]. Proteoglycan synthesis was examined by labelling the cultures with $^{35}SO_4$ followed by analysis of the secreted products by SDS-PAGE and autoradiography as described[9,17]. Markers were myosin and β-galactosidase with relative molecular masses (M_r) of 200,000 and 116,000, respectively ($M_r \times 10^{-3}$). *b*, Total RNA was prepared by lysis of isolated glomeruli in guanidine isothiocyanate and ultracentrifugation of lysate on a caesium chloride cushion[33]. Samples of 10 μg were electrophoresed in a 2.2 M formaldehyde/1% agarose gel and transferred to a nitrocellulose membrane. The membranes were prehybridized for 5 h at 37 °C in 5 × SSC, 5 × Denhardt's solution, 0.1 mg ml^{-1} salmon sperm DNA, 0.1% SDS and 50% formamide. A porcine TGF-β1 complementary DNA probe[34] and a rat glyceraldehyde 3-phosphate dehydrogenase cDNA probe[35] were labelled with [^{32}P]CTP by the random primer method and hybridized at 42 °C for 15 min. The TGF-β1 mRNA result is shown; the amount of the dehydrogenase mRNA was equal in all samples and is not shown.

is likely to be TGF-β1 because the antiserum was prepared against a peptide from this TGF-β. Because the amino-acid sequence of the various TGF-βs are similar[14,24], however, the antiserum could also have affected other types of TGF-β.

The mechanism of TGF-β1 action is likely to be the extremely strong stimulation of extracellular matrix synthesis, manifested as a marked induction of proteoglycan synthesis by TGF-β1 in cultural mesangial cells and of proteoglycan, fibronectin and other matrix molecules in cultured glomerular epithelial cells[17,25]. The ability of TGF-β1 to suppress the expression of proteases and stimulate the expression of protease inhibitors[26,27] may also contribute to the accumulation of extracellular matrix.

We have used antithymocyte serum to produce acute mesangial matrix expansion *in vivo*. Although the aetiological mechanism in man is not known, accumulation of mesangial matrix is also a prominent and important pathological feature of human mesangial proliferative glomerulonephritis[28] and diabetic nephropathy[32]. The impressive suppression of the experimental disease achieved with anti-TGF-β1 treatment indicates the importance of TGF-β in regulating extracellular matrix in glomerulonephritis. This suggests that TGF-β may have a similar role in human glomerular diseases, and perhaps other diseases in which fibrosis is a factor. □

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Voltage-dependent $InsP_3$-insensitive calcium channels in membranes of pancreatic endoplasmic reticulum vesicles

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STIMULUS–SECRETION coupling in exocrine glands involves Ca^{2+} release from intracellular stores[1,2]. In endoplasmic reticulum vesicle preparations from rat exocrine pancreas, an inositol 1,4,5-trisphosphate($InsP_3$)-sensitive, as well as an $InsP_3$-insensitive, Ca^{2+} pool has been characterized[3]. But Ca^{2+} channels in the endoplasmic reticulum of rat exocrine pancreas have not been demonstrated at the level of single-channel current. We have now used the patch-clamp technique on endoplasmic reticulum vesicles fused by means of the dehydration–rehydration method[4–6]. In excised patches, single Ba^{2+}- and Ca^{2+}-selective channels were recorded. The channel activity was markedly voltage-dependent. Caffeine increased channel open-state probability, whereas ruthenium red and Cd^{2+} blocked single-channel currents. Ryanodine, nifedipine and heparin had no effect on channel activity. The channel activity was not dependent on the free Ca^{2+} concentration, the presence of $InsP_3$, or pH. We conclude that this calcium channel mediates Ca^{2+} release from an intracellular store through an $InsP_3$-insensitive mechanism.

The endoplasmic reticulum (ER) of rat exocrine pancreas is known to play a central part in the regulation of cytoplasmic free Ca^{2+} concentration[1,7,8]. To investigate whether Ca^{2+} channels are involved in the Ca^{2+}release mechanisms, isolated ER vesicles were fused to giant vesicles (10–100 μm) by the dehydration–rehydration method[4–6]. These giant ER vesicles were used for studies with the patch-clamp technique. To avoid current fluctuations from the large conductance anion channel[5], Cl^- was replaced by HEPES buffer in both bath and pipette solutions. With K^+ buffer (75 mM KOH, 280 mM HEPES buffer, pH 7.1) in the pipette and Ba^{2+} buffer (50 mM $Ba(OH)_2$, 280 mM HEPES buffer, pH 7.3) in the bath, single-channel currents carried by Ba^{2+} ions flowing from the bath into the pipette were recorded in 87% of stable seals. The mean channel conductance was 47±3 pS ($n = 19$). Replacement of Ba^{2+} by Ca^{2+} showed that the channel is also permeable to Ca^{2+} ions. A single membrane patch contained 1–4 (usually 1 or 2) channels.

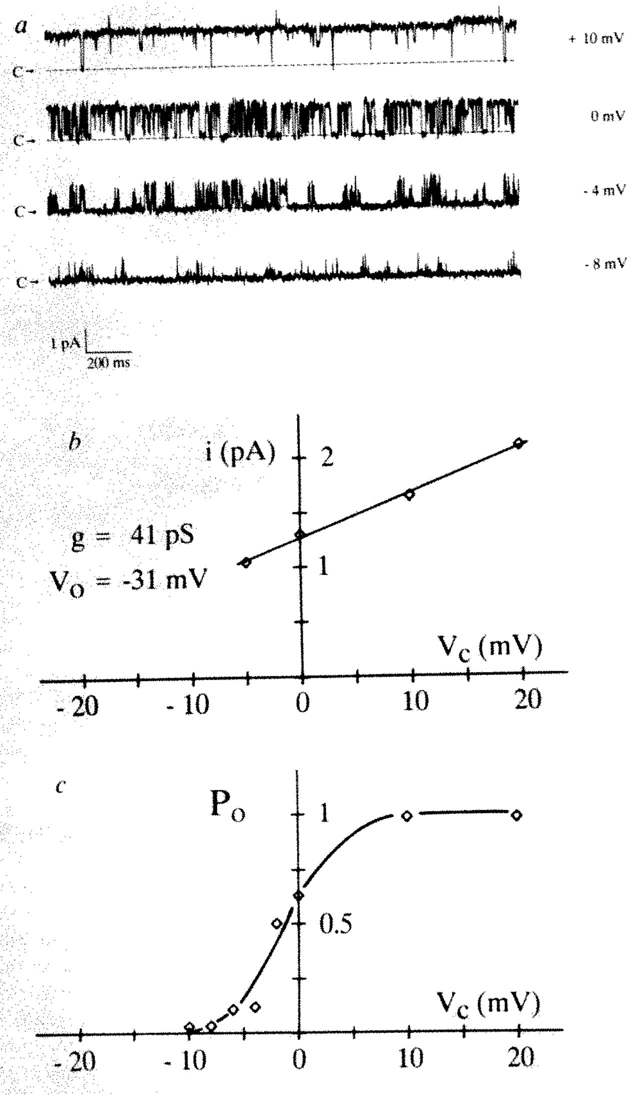
Typical single-channel current traces are shown in Fig. 1*a*. The conductance of this channel determined by linear regression was 41 pS (Fig. 1*b*). From the extrapolated reversal potential of −31 mV, a permeability ratio $P_{Ba^{2+}}/P_{K^+}$ of ~15:1 is estimated. The channel activity displayed a distinct voltage-dependence (Fig. 1*a* and *c*). Positive clamp voltages (+10 to +20 mV; sign refers to bath side) increased channel open-state probability, whereas negative potentials led to channel inactivation. In addition to the full current amplitude, sublevels of conductance occurred (Fig. 1*a*). Frequency, duration and magnitude of the sublevels varied in different experiments. As well as the voltage-dependent inactivation, in most experiments a spontaneous run-down of channel activity occurred with time; channel activity could be restored by applying short voltage pulses (±40 mV).

To further characterize the channel, several potential Ca^{2+} channel activators and blockers were tested. Ryanodine[9,10] (pipette 200 μM, $n = 11$; bath 50 μM, $n = 5$), which is known to arrest the Ca^{2+} release channel from skeletal muscle sarcoplasmic reticulum (SR) on a sublevel of conductance, had no effect on Ca^{2+} channels in pancreatic ER membranes. Neither heparin (100 μg ml^{-1}, $n = 7$), an inhibitor, nor $InsP_3$ (10–50 μM, $n = 10$), an activator of $InsP_3$-sensitive Ca^{2+} channels in smooth muscle SR[11], nor nifedipine (10–100 μM, $n = 9$), a blocker of voltage-dependent dihydropyridine-sensitive L-type calcium channels[12], had any detectable effects on either side of the membrane patch. The Ca^{2+} channel in fused pancreatic ER membranes, therefore, clearly has different pharmacological properties from Ca^{2+} channels in other tissues.

In contrast to skeletal muscle SR calcium channels[13], the activity of pancreatic ER calcium channels is independent of Ca^{2+} concentration. Thus, removal of Ca^{2+} ions from the pipette (5 mM EGTA; free $Ca^{2+} < 10^{-9}$ M; $n = 9$) did not suppress channel activity, and neither elevated Ca^{2+} concentrations on the pipette side (free Ca^{2+} 10^{-3} M; $n = 19$), nor addition of Ca^{2+} ions (free Ca^{2+} 10^{-5} M; $n > 20$) to the bath solution influenced channel appearance. Channel activity was also unaffected by changes in pH on the pipette side from pH 6.7 (40 mM KOH, 320 mM HEPES buffer; $n = 8$) to pH 7.8 (115 mM KOH, 180 mM HEPES buffer; $n = 6$).

Caffeine (10 mM), which is known to activate cardiac SR Ca^{2+} channels[14], also increased activity of Ca^{2+} channels in pancreatic ER membranes (Fig. 2*b*). This effect was, however, only observed in 4 out of 10 experiments. Ruthenium red (10 μM), an inhibitor of skeletal SR Ca^{2+} channels[15] reduced Ca^{2+} channel activity (Fig. 2*c*).

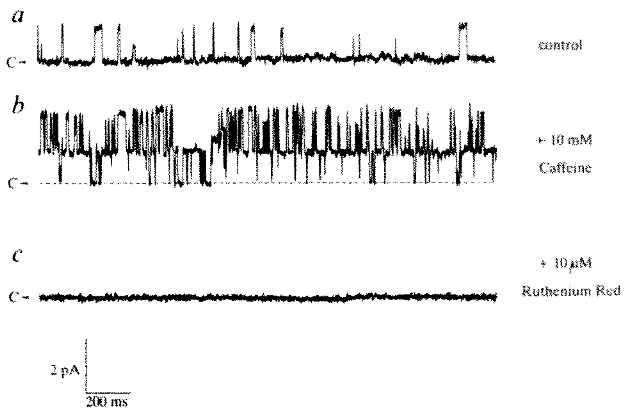
The Ca^{2+} channels in ER membranes can also be blocked by



◀ FIG. 1 *a*, Single-channel currents in a membrane patch excised from a giant vesicle produced by fusion of ER vesicles from rat pancreatic acinar cells. C→ marks the baseline current (closed state). Channel openings are shown by upward currents, indicating flow of Ba^{2+} ions from the bath into the pipette. The clamp potentials on the right refer to the bath. The channel activity is markedly voltage-dependent: at +10 mV the channel is open most of the time, but even small negative clamp potentials lead to drastic reduction of channel open times and increase of closed times. At −8 mV channel openings are only visible as short current spikes. The corresponding current-voltage data are shown in *b*. The data points are fitted to a straight line. Extrapolation to the voltage axis yields a reversal potential (E_{rev}) of −31 mV. *c*, The channel open state probability (P_O) versus the clamp potential (V_c). The curve was fitted by eye.

METHODS. ER vesicles were prepared from isolated rat exocrine pancreas cells[23]. Cells were homogenized in 280 mM mannitol, 10 mM KCl, 5 mM HEPES buffer, 1 mM $MgCl_2$, 1 mM benzamidine, pH 7.0 with Tris buffer. Cell debris, nuclei and mitochondria were removed from the homogenate by centrifugation at 400*g* and 11,000*g* (15 min). The resulting supernatant was centrifuged at 27,000*g* for 15 min, the pellet resuspended and adjusted to protein concentrations of 10–20 mg ml^{-1}. Vesicle fusion was performed with the dehydration-rehydration technique[5,6]; droplets (10 μl) of the vesicle suspension were dehydrated in a desiccator by vacuum evaporation (10–20 min) and K^+-HEPES buffer (75 mM KOH, 280 mM HEPES, pH 7.1) added for rehydration. Fusion to giant vesicles occurred within 0.5–2 h. Clusters of the still aggregated, rehydrating vesicles were transferred into the measuring chamber and allowed to settle for 10–30 min, then the K^+-HEPES solution was carefully replaced by a Ba^{2+}-HEPES solution (50 mM $Ba(OH)_2$, 280 mM HEPES, pH 7.3). Measurements were carried out at room temperature (20–22 °C). The tip of the patch pipette was filled with K^+-HEPES buffer overlain with 140 mM KCl solution. The bath was connected by an agar bridge to a Ag/AgCl electrode in 140 mM KCl. Clamp voltages were corrected for the reversal potential of the respective baseline current, which in most experiments was within the range 0 to +5 mV. $P_{Ba^{2+}}/P_{K^+}$ was calculated according to the constant field theory: $P_{Ba^{2+}}/P_{K^+} = a_K \cdot (exp^u + 1)/4 \cdot a_{Ba} \cdot exp^{2u}$, where $u = F \cdot E_{rev}/R \cdot T$, and assuming activity coefficients of about 0.78 for γ_{K^+} and about 0.29 for $\gamma_{Ba^{2+}}$[24]. Further details about patch-clamp recording technique and data analysis were described previously[25].

FIG. 2 Activity of Ca^{2+} channels in membranes of fused ER vesicles can be increased by caffeine. C→ marks the closed state. Channel openings are shown by upward currents. The clamp potential was −10 mV. Under control conditions channel openings occurred in short sporadic spikes (*a*). Sixty seconds after addition of caffeine (10 mM) to the bath, activity of two channels with increased open probability was observed (*b*). A similar increase in channel activity was never observed under control conditions. Addition of 10 μM ruthenium red to the bath solution led to complete inactivation of the Ca^{2+} channels (*c*). Channel activity could be restored by bath perfusion with control solution. In some experiments, when ruthenium red was added to the bath solution, only incomplete channel blockage was observed. The blocking effect was more pronounced when ruthenium red was applied from the pipette side. As fluid exchange in the pipette was not feasible because of poor stability of the membrane seals, different membrane patches have to be compared. Under control conditions, in 87% of stable seals ($n = 60$) Ca^{2+} channels were detectable. With 10 μM ruthenium red in the pipette solution single calcium channels retained activity in only 2 out of 20 experiments. In all other cases channel activity was still observed during the first 1–2 seconds after seal formation but then disappeared completely. ▶



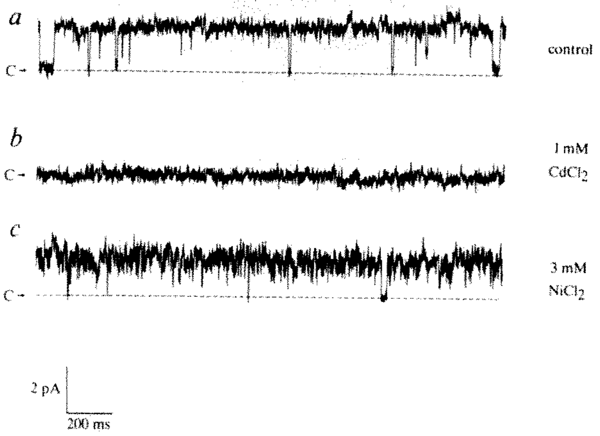


FIG. 3 Effects of Cd^{2+} and Ni^{2+} on Ca^{2+} channel activity in pancreatic ER membranes. *a*, Normal channel activity under control conditions. V_c was +5 mV. *b*, Addition of 1 mM Cd^{2+} to the bath solution resulted in rapid and complete channel blockage. In contrast, 3 mM Ni^{2+} did not suppress channel activity but induced rapid flickering with decrease in mean current amplitude (*c*). The effects of both Cd^{2+} and Ni^{2+} were fully reversible.

addition of Cd^{2+} to the bath (Fig. 3*b*), as observed for Ca^{2+} channels in heart ventricular cells[16]. Complete channel blockage was achieved by application of 500 μM Cd^{2+} (0.5-1 mM; $n = 9$). Ni^{2+} was a less potent inhibitor (Fig. 3*c*); millimolar concentrations (1-3 mM) induced rapid flickering but did not suppress channel activity ($n = 7$). These effects were fully reversible.

The voltage-dependent Ca^{2+} channels described in this study were neither dependent on the presence of $InsP_3$, nor was channel activity suppressed by heparin. We therefore conclude that these channels are not involved in the $InsP_3$-triggered Ca^{2+} release mechanism in pancreatic ER membranes. However, we have never observed $InsP_3$-induced Ca^{2+} channels, as had been found in smooth muscle SR vesicles[11]; $InsP_3$-induced Ca^{2+} channels in the ER of the exocrine pancreas of the rat could be too small to be detected (<5 pS).

Recent studies of several cell types provide evidence for the existence of more than one non-mitochondrial intracellular Ca^{2+} store and Ca^{2+} release mechanism[3,17-20]. For example, Ca^{2+}-induced Ca^{2+} release similar to that in skeletal muscle SR was implicated in rat lacrimal gland cells[17]. However, the Ca^{2+} channels found in rat exocrine pancreas ER membranes are clearly different from SR Ca^{2+} channels. The conductance is smaller, a ryanodine effect could not be observed and calcium on the pipette side—which is assumed to represent the cytosolic membrane side—has no activating effect. Thus, we conclude that the voltage-dependent Ca^{2+} channels described in this study are not identical to the Ca^{2+} release mechanism observed in SR membranes. Studies on Ca^{2+} uptake into ER vesicles from rat exocrine pancreas revealed at least two distinct Ca^{2+} pools, an $InsP_3$-sensitive and an $InsP_3$-insensitive one[3]. Further studies showed that Ca^{2+} ions can be released from an $InsP_3$-insensitive pool by caffeine (M.D.-K. and I.S., manuscript in preparation). The type of voltage-dependent Ca^{2+} channel described in this study is probably located in this caffeine-sensitive Ca^{2+} pool. The most striking feature of the Ca^{2+} channel in ER membranes is its voltage-dependence, which means that Ca^{2+} release from the vesicles would be inhibited by inside negative voltages. Current models for function of $InsP_3$-sensitive and $InsP_3$-insensitive Ca^{2+} pools involve GTP-induced connection between different Ca^{2+} pools[21,22]. One possible physiological signal inducing Ca^{2+} release through voltage-dependent Ca^{2+} channels could therefore be linkage of Ca^{2+} channel-containing vesicles to other vesicles that possess either an inside positive potential or ion permeabilities short-circuiting the membrane potential. □

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Escape of DNA from mitochondria to the nucleus in *Saccharomyces cerevisiae*

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THE migration of genetic information from ancestral prokaryotic endosymbionts into eukaryotic nuclei is thought to have had an important role in the evolution of mitochondria and chloroplasts[1-6]. Here we describe an assay for the detection of movement of DNA between mitochondria and the nucleus in yeast. Because recombinant plasmid DNA replicates after transformation into mitochondria of yeast strains lacking endogenous mitochondrial DNA[7] we were able to propagate the nuclear genetic marker *URA3* in mitochondria. As expected, the wild-type *URA3* gene in mitochondria failed to complement the uracil auxotrophy (Ura$^-$) caused by a nuclear *ura3* mutation. But selection of Ura$^+$ prototrophs from a Ura$^-$ strain carrying *URA3* on a plasmid in its mitochondria enabled us to detect plasmid movement to the nucleus. Conversely, as the plasmid used also contained the mitochondrial gene *COX2* required for respiratory growth, we were able to set up corresponding selections to detect migration of DNA from the nucleus to the mitochondria. Our results show that, in yeast, DNA escapes from mitochondria and appears in the nucleus at a surprisingly high frequency ($\sim 2 \times 10^{-5}$ per cell per generation). But the rate at which DNA makes the journey in the opposite direction—nucleus to mitochondria—is apparently at least 100,000 times less.

A yeast strain with mitochondria that contained only sequences derived from a defined plasmid was generated using high velocity microprojectile bombardment[7-9]. If a ρ^0 yeast strain, lacking endogenous mitochondrial DNA (mtDNA), is bombarded with a plasmid carrying the mitochondrial gene *COX2* (previously referred to as *oxi1* (refs 10, 11)), rare transformants can be isolated in which the plasmid is present in mitochondria[7]. These transformants (synthetic ρ^- strains) mimic the behaviour of natural ρ^- strains, which lack a mitochondrial translation system and thus fail to respire. But as the transformants contain many copies of the *COX2* gene in mitochondria they can, like natural ρ^- strains retaining *COX2*, complement the respiratory defect of a ρ^+ strain (that retains mitochondrial translation) with a *cox2* deletion. The ability to complement is evidence that

TABLE 1 Yeast strains

Strain	Nuclear genotype	Mitochondrial genotype
F189ρ^+	*MAT***a**, *ade2-101, ura3-52*	ρ^+
F189ρ^0	*MAT***a**, *ade2-101, ura3-52*	ρ^0
PTY1	*MAT***a**, *ade2-101, ura3-52,* [*URA3* (pMK2)]	ρ^-, *COX2* (pMK2)
PTY2	*MAT***a**, *ade2-101, ura3-52*	ρ^-, *COX2*, (pMK2)
PTY3	*MAT***a**, *ade2-101, ura3-52,* [*URA3* (pMK2)]	ρ^0
PTY6	*MAT*α, *ade2, ura3-52*	ρ^+, *cox2-17*
PTY16	*MAT***a**, *ino1, can*^{r}, *ura3-52*	ρ^-, *COX2* (pMK2)
PTY17	*MAT***a**, *trp1-Δ1, ura3-52*	ρ^-, *COX2* (pMK2)
PARB1	*MAT*α, *ade2-101, ura3-52*	ρ^+, *cox2-17*

Yeast strains TF189ρ^+, TF189ρ^0, PTY1, PTY2, PTY3 and PARB1 are all sogenic to DBY947 (ref. 25). PARB1 was obtained from P. Anziano and R. A. Butow. PTY16 was derived from strain TD28ρ^0 (ref. 7). PTY17 was derived from D273-10B (ATCC25657). The *cox2-17* allele of PTY6 is a complete deletion of the *COX2* coding region of mtDNA, previously termed *oxi1-17* (ref. 7). Strain PTY1 was made by bombardment of TF189ρ^0 with pMK2-coated tungsten particles essentially as described[7]. pMK2 DNA (17 μg) were precipitated on to 3 mg of 1.2-μm tungsten particles in the presence of 1.25 M $CaCl_2$ and 10 mM spermidine. One fifth (0.6 mg) of these DNA-coated tungsten particles were used to bombard a minimal glucose–adenine agar plate (lacking uracil) spread with about 1×10^8 cells. After bombardment of 40 plates, ~2,000 nuclear transformants (Ura$^+$ cells) were screened for the presence of pMK2 DNA in the mitochondria by mating with a ρ^+, *cox2-17* tester and subsequent screening for respiring diploids. PTY3 was a nuclear transformant picked from a bombarded lawn that failed to complement the ρ^+, *cox2-17* tester. PTY2 was a mitotic segregant of PTY1 that had lost pMK2 from the nucleus. PTY16 and PTY17 were constructed by transfer of ρ^- [pMK2] mitochondria into ρ^0 strains with the corresponding nuclear genetic backgrounds. The mitochondria were passaged through a ρ^0 derivative of the *kar1-1* yeast strain, 8960-11B (obtained from G. R. Fink).

the transformants contain the plasmid in mitochondria because, as noted previously[7] and confirmed below, mitochondrial genes on plasmids present in the yeast nucleus are genetically inactive.

Our experiment required a recombinant plasmid whose presence could be detected in either the nuclear or mitochondrial compartments. The plasmid pMK2 contains the *URA3* gene[12], a common nutritional marker for nuclear transformation in yeast, and the mitochondrial marker *COX2* which encodes subunit II of cytochrome oxidase. In addition, pMK2 contains the origin of replication of the yeast 2μ plasmid, allowing autonomous propagation in the nucleus. There is apparently no specific sequence required for replication of DNA in the mitochondria of ρ^- yeast[13].

The yeast strain TF189ρ^0 (Table 1), a uracil auxotroph by virtue of the nonreverting *ura3-52* allele[14], was bombarded by particles coated with pMK2 DNA[7]. One strain recovered, designated PTY1 (Table 1), had pMK2 DNA in its mitochondria, judged on the basis of its ability to complement a *cox2* deletion mutant after mating. A Ura$^-$ mitotic segregant of PTY1, named PTY2, was isolated and shown to contain pMK2 sequences, unrearranged except for the expected presence of a high proportion of concatemers characteristic of ρ^- mtDNA[7,15] (Fig. 1). PTY2, like many natural ρ^- strains[15], also contained monomer circles. PTY2 was auxotrophic for uracil despite the fact that it contained a *URA3* gene, because that gene was located in mitochondria, not in the nucleus. (As a ρ^- strain lacking mitochondrial protein synthesis, PTY2 cannot express *URA3*.) Figure 2*a* is a schematic depiction of PTY2, a strain with copies of a nuclear gene located in mitochondria that are potentially capable of complementing a nuclear mutation.

Several clones of PTY2 were grown on complete medium agar plates at 30 °C. Individual clones were excised, suspended in water and transferred to agar plates lacking uracil in order to select cells that had become Ura$^+$ despite carrying the nonreverting nuclear *ura3-52* mutation. Numerous Ura$^+$ colonies were observed from every ρ^- clone. Using the statistical analysis of Luria and Delbrück[16] we found that PTY2 yielded Ura$^+$ prototrophs at a rate of $\sim 2 \times 10^{-5}$ per cell generation under these conditions (Table 2).

The appearance of Ura$^+$ prototrophs depended strictly on the presence of pMK2 DNA in the mitochondria. More than 50 mitotic segregants of PTY2 were isolated that no longer contained pMK2 DNA in their mitochondria, as judged by their inability to complement a *cox2* deletion. In every case, these segregants failed to give rise to Ura$^+$ prototrophs. This strong linkage between *COX2* and *URA3* confirmed that the source of genetic information allowing complementation of the *ura3-52* mutation in PTY2 was pMK2 DNA contained within the mitochondria.

The Ura$^+$ derivatives selected from PTY2 behaved as though pMK2 was replicating as a plasmid in their nuclei. First, the Ura$^+$ phenotype was mitotically unstable, as expected from a 2μ-based plasmid[17]. Second, DNA was isolated from several Ura$^+$ prototrophs, as well as from strains derived from these prototrophs that had lost pMK2 from the mitochondria. Gel-blot hybridization analysis revealed that the pMK2 plasmid sequences transferred to the nucleus had not been rearranged during

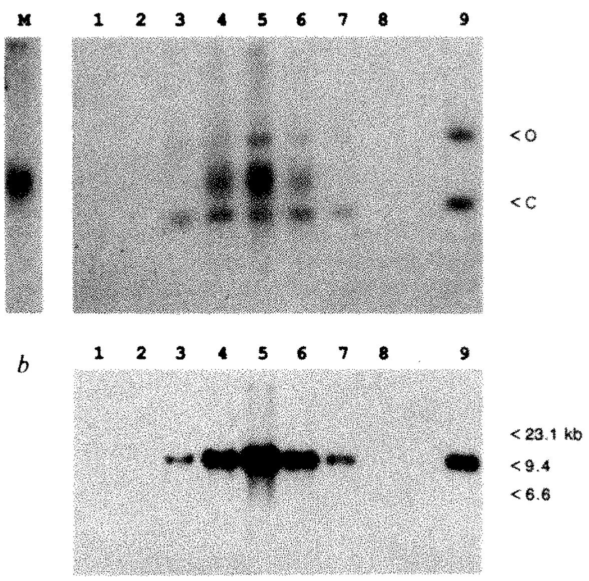

FIG. 1 Gel-blot-hybridization analysis of DNA from yeast strains containing pMK2 in their nuclei and/or mitochondria. *a*, Undigested DNAs: Total cellular DNA from strains TF189ρ^+ (lane 1), TF189ρ^0 (lane 2), PTY3 (lane 3), PTY1 (lane 4), PTY2 (lane 5), a Ura$^+$, ρ^- derivative of PTY2 (lane 6), a Ura$^+$, ρ^0 derivative of PTY2 (lane 7), a Ura$^-$, ρ^0 derivative of PTY2 (lane 8) and CsCl gradient purified pMK2 (lane 9) were run on an agarose gel and blotted to a filter. Samples in lanes 1–9 were probed with ^{32}P-labelled pBR322 DNA (the backbone of plasmid pMK2) and autoradiographed. The positions of open (O) and closed (C) circular pMK2 are indicated. In lane M, DNA from TF189ρ^+ was run and probed with yeast [^{32}P] mtDNA purified on a CsCl gradient. The broad band of mtDNA in lane M is at the same position as the signals corresponding to multimeric plasmid DNA in strains containing pMK2 in their mitochondria (lanes 4, 5 and 6). *b*, DNAs digested with *Eco*RI: lanes 1–9 correspond to *a*, except that the DNAs were digested with *Eco*RI before electrophoresis and the filter probed with ^{32}P-labelled pMK2 DNA. In every case, plasmid DNA was reduced to the same size as purified pMK2 (lane 9). DNA isolation, electrophoresis in 1% agarose containing Tris-phosphate buffer with 1 μg ml^{-1} ethidium bromide, blotting and hybridization were carried out as previously described[26–28]. Abbreviation: kb, kilobases.

TABLE 2 Rate of appearance of uracil prototrophs in strains with pMK2 in their mitochondria

Strain	Growth temperature (°C)	Events per cell per generation
PTY2	30	2×10^{-5}
PTY16	30	9×10^{-5}
PTY17	30	4×10^{-5}
PTY17	37	10×10^{-5}
PTY17	14	8×10^{-5}

Cells grown in complete medium were either micromanipulated or diluted and spread on to complete-medium agar plates to obtain colonies derived from single cells. Between 10 and 20 independent colonies were examined for each strain. After growth for several days at the indicated temperature, whole colonies (about 5×10^6 cells) were excised and resuspended in 1 ml sterile water. The total cells per colony and Ura$^+$ cells per colony were determined by plating aliquots on complete medium, and medium lacking uracil, respectively. In each case, the rate at which uracil prototrophs arose was determined numerically using the equation derived by Luria and Delbrück[16], $r=aN_t \ln(N_tCa)$ where r is the observed average number of Ura$^+$ cells per colony, C is the number of colonies examined, N_t is the total number of cells in a colony, and a is the number of Ura$^+$ events per time unit. The rates reported above are the value of a multiplied by ln 2.

their propagation or migration (Fig. 1). Indeed, the DNA from the Ura$^+$ prototrophs derived from PTY2 by escape of pMK2 from mitochondria to the nucleus was indistinguishable from that of the original transformant PTY1.

These results strongly support the notion that a cytological event allows pMK2 to escape from mitochondria and enter the nucleus (Fig 2*a*, *c*), and that this event does not involve a genetic mutation. As further confirmation of this idea, we repeated the entire series of experiments in cycles starting with Ura$^-$ mitotic segregants selected from the Ura$^+$ derivatives of PTY2. We were able repeatedly to isolate spontaneous Ura$^+$ derivatives from Ura$^-$ mitotic segregants, and the process remained strictly dependent on the maintenance of pMK2 sequences in mitochondria.

To test whether the frequency of pMK2 escape from mitochondria would vary in strains with different nuclear genetic backgrounds, the ρ^- mitochondria of strain PTY2 were transferred to ρ^0 derivatives of two other yeast strains, using a *kar1* mutant[18] (Table 1). (The ability to complement *cox2* remained completely linked to the ability to become Ura$^+$ during thes transfers, further supporting the notion that *URA3* was locate in mitochondria.) The rate of pMK2 escape from mitochondri of PTY17 was twofold greater, and of PTY16 more than fourfol greater, than that of PTY2 (Table 2), indicating that geneti background affects the process that allows DNA to escape fro mitochondria and migrate to the nucleus.

The rate of escape was also affected by temperature and th composition of the medium. Cells grown at either low (14 °C or high (37 °C) temperature yielded more Ura$^+$ segregants tha cells grown under optimal conditions (30 °C) (Table 2). Furthermore, when cultures of PTY17 were frozen at −70 °C in wate containing 15% glycerol, thawed and replated, the number o Ura$^+$ derivatives increased threefold over that obtained by plating an unfrozen aliquot of the culture incubated at room temperature in glycerol for the same brief period. Incubation o PTY17 in 15% glycerol at 30 °C for 6 hours, however, resulte in a threefold increase in the proportion of Ura$^+$ cells whe compared with cells from the same culture incubated in water (Incubation in water had no effect on the number of Ura$^+$ cell in the sample.)

Escape of DNA from mitochondria occurs not only in respiratorily defective ρ^- cells, but also in respiring ρ^+ cells. W showed this by selecting respiring Ura$^+$ diploids from Ura$^-$ zygotic clones, generated by mating PTY2 with PTY6. Th recovery of respiring cells, carrying *URA3* on a plasmid in th nucleus and expressing the plasmid-derived *COX2* gene i mitochondria (not shown), demonstrated escape in respiring cells. These diploids were heteroplasmic for two forms o mtDNA, and no calculation of the rate of escape could be made owing to the instability of the heteroplasmic state.

In light of the evidence demonstrating the migration of DNA from mitochondria to the nucleus at high frequency, we examined the possibility that movement might also occur in the reverse direction. We isolated a Ura$^+$-transformed strain, PTY3 (Table 1), that contained the plasmid pMK2 only in the nucleus (shown schematically in Fig. 2*b*). If pMK2 could migrate from the nucleus into the mitochondria of this ρ^0 strain, then PTY3 would be expected to give rise to ρ^- derivatives (shown schematically in Fig. 2*c*) capable of complementing the *cox2* deletion of the ρ^+ strain PTY6 (Table 1). PTY3 was grown selectively to maintain the plasmid and $\sim10^8$ cells were mated with an equal number of PTY6 cells. None of the resulting diploids (at least 5×10^7) gave rise to respiring colonies. So,

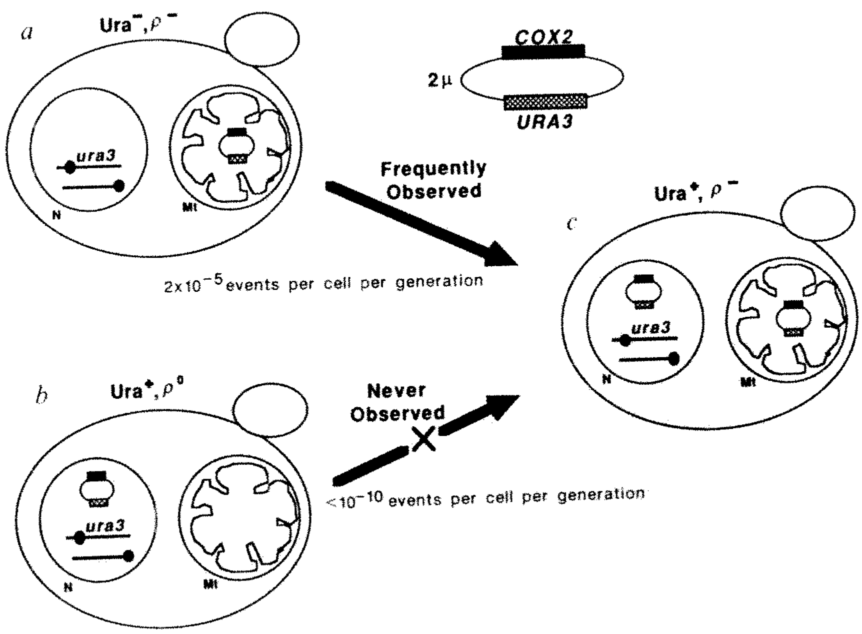

FIG. 2 Experimental scheme. A plasmid carrying the nuclear gene *URA3*, the 2μ origin of replication and the mitochondrial gene *COX2* (pMK2) could be maintained in either the nucleus (N), the mitochondrion (Mt) or both compartments. *a*, Cells containing the plasmid in their mitochondria (equivalent to the yeast strain PTY2 (Table 1)) frequently give rise to cells containing the plasmid in both the nucleus and mitochondria, C. *b*, Cells containing the plasmid in the nucleus (equivalent to the yeast strain PTY3 (Table 1)) failed to give rise to cells with the plasmid in both compartments, C, at detectable frequency. As indicated, all strains carried a nonreverting chromosomal *ura3* mutation.

nder these conditions, the movement of DNA from the nucleus o the mitochondria was at least 100 times less frequent than ne observed movement in the opposite direction.

To select more strongly for movement from the nucleus to mitochondria, we directly transformed the nucleus of strain ARB1, a *ura3-52*, ρ^+ strain carrying the *cox2* deletion mutation Table 1), with pMK2. The only means by which this nonrespiring transformant could give rise to respiring cells would be by he transfer of pMK2 sequences that contained the *COX2* gene rom the nucleus to mitochondria. About 10^{10} cells were grown rom this transformant under conditions selecting for maintenance of pMK2 in the nucleus and plated on complete media hat required respiration for growth. No respiring colonies were observed. Thus, under conditions that allowed frequent transfer of DNA from mitochondria to the nucleus, no transfer of DNA rom the nucleus to mitochondria was detected.

The mechanism by which DNA moves from mitochondria to nuclei is unknown. Uptake of DNA from the cytoplasm into nuclei seems to be a very general phenomenon. Yeast nuclei can be transformed by a variety of procedures[7,9,19-22] that presumably introduce DNA from outside the cell into the cytoplasm. The nuclei can take up DNA molecules ranging in size from small plasmids to chromosome-sized pieces[23]. Indeed, internuclear transfer of chromosomes has been observed in yeast heterokaryons[24]. So how does DNA escape from mitochondria to the cytoplasm? One possibility is that transient breaches in mitochondrial membranes allow the release of mtDNA from the matrix. The increase in DNA migration observed in cells grown at high or low temperature, subjected to freezing or incubation with glycerol—actions that might disrupt mitochondrial membranes—is consistent with this hypothesis. Another possibility is that the escape events observed in these studies were initiated during terminal degradation of organellar structures that occasionally released intact DNA molecules. This hypothesis would explain the failure to observe plasmid migration into mitochondria. In any event, the assay described here for the movement of DNA within a eukaryotic cells makes possible further physiological and genetic studies that may define molecular mechanisms important in compartmentalization and organization of nuclei and mitochondria. ☐

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Triggering of cyclin degradation in interphase extracts of amphibian eggs by cdc2 kinase

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THE cell cycles of early *Xenopus* embryos consist of a rapid succession of alternating S and M phases[1]. These cycles are controlled by the activity of a protein kinase complex (cdc2 kinase) which contains two subunits. One subunit is encoded by the frog homologue of the fission yeast *cdc2*$^+$ gene, $p34^{cdc2}$ (ref. 2) and the other is a cyclin[3]. The concentration of cyclins follows a sawtooth oscillation because they accumulate in interphase and are destroyed abruptly during mitosis[3]. The association of cyclin and $p34^{cdc2}$ (refs 4–7) is not sufficient for activation of cdc2 kinase, however; dephosphorylation of key tyrosine and threonine residues of $p34^{cdc2}$ is necessary to turn on its kinase activity[8–11]. The activity of cdc2 kinase is thus regulated by a combination of translational and post-translational mechanisms. The loss of cdc2 kinase activity at the end of mitosis depends on the destruction of the cyclin subunits[3,4,12,13]. It has been suggested that this destruction is induced by cdc2 kinase itself, thereby providing a negative feedback loop to terminate mitosis[14,15]. Here we report direct experimental evidence for this idea by showing that cyclin proteolysis can be triggered by adding cdc2 kinase to a cell-free extract of interphase *Xenopus* eggs.

To study the control of cyclin degradation, [^{35}S]methionine-labelled sea urchin cyclin was made in a reticulocyte lysate programmed with synthetic messenger RNA[16]. A portion of the synthesis mix was added to an interphase extract prepared from activated *Xenopus* eggs[15]. Cyclin stability was monitored by gel electrophoresis and autoradiography and quantified by scanning densitometry. Labelled cyclin was reasonably stable in interphase extracts, but when $12\ \mathrm{U}\ \mu\mathrm{l}^{-1}$ of active cdc2 kinase was added, cyclin was rapidly destroyed after a 25-min lag (Fig. 1*a*). Raising the level of cdc2 kinase to $24\ \mathrm{U}\ \mu\mathrm{l}^{-1}$ reduced this lag to about 15 min, but did not increase the rate of destruction once it had started. Further increase in the level of cdc2 kinase did not markedly shorten the lag phase or affect the kinetics of cyclin destruction. These results suggest that cdc2 kinase initiates a chain of events, culminating in cyclin destruction. It is noteworthy that the minimum level of cdc2 kinase required to trigger cyclin destruction in these extracts ($12\ \mathrm{U}\ \mu\mathrm{l}^{-1}$) is close to what is found during mitosis *in vivo*[17] and in M-phase extracts[15]. The induction of cyclin proteolysis by cdc2 kinase required the presence of *Xenopus* extract. It did not occur when the extract was replaced with buffer (Fig. 1*a*), nor when it was replaced by the rabbit reticulocyte lysate in which the cyclin had been synthesized (data not shown). When the *Xenopus* extract was diluted two- or fourfold, the rate of cyclin destruction was reduced but the length of the lag was essentially unaffected. A 10-fold-diluted extract did not support cyclin destruction (Fig. 1*a*).

These experiments used preparations of cdc2 kinase purified by $p13^{suc1}$ affinity chromatography as an equimolar complex of $p34^{cdc2}$ and cyclin[6] (Fig. 1*b*, lane 1). It was therefore possible that the proteolytic pathway was activated by the added cyclin component, and not by $p34^{cdc2}$. To examine this possibility, we repeated the experiments using a preparation of cdc2 kinase lacking intact cyclin subunits[18] (Fig. 1*b*, lane 2). This form of the enzyme induced cyclin degradation as well as did cdc2 kinase with intact cyclin, and the same level of kinase activity was required (Fig. 1*c*). Therefore, it is unlikely that a threshold level of cyclin *per se* is the trigger for proteolysis.

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It was possible that the added cdc2 kinase promoted cyclin degradation by activating the latent endogenous cdc2 kinase activity of the extract[19]. This did not seem to be the case, however, because during these incubations, there was no increase in the level of histone H1 kinase activity over and above the level attributable to the added cdc2 kinase. Indeed, an abrupt loss of kinase activity occurred concomitant with cyclin degradation (Fig. 2*a*). So addition of exogenous cdc2 kinase did not lead to detectable activation of the endogenous cdc2 kinase activity in these crude extracts. This contrasts with the finding of Dunphy and Newport[19] who showed that the activity of maturation-promoting factor (MPF), assayed by the ability of MPF to trigger nuclear envelope breakdown in extracts of interphase *Xenopus* eggs, could be amplified in ammonium sulphate fractions of similar extracts. The two approaches differ in significant ways, however. We added highly purified cdc2 kinase to total cell extracts, whereas they used partially purified MPF added to an ammonium sulphate fraction of similar extracts. Furthermore they included ATP-γ-S in the incubation, which we did not. Finally, and perhaps most significantly, they were measuring MPF and not H1 kinase activity.

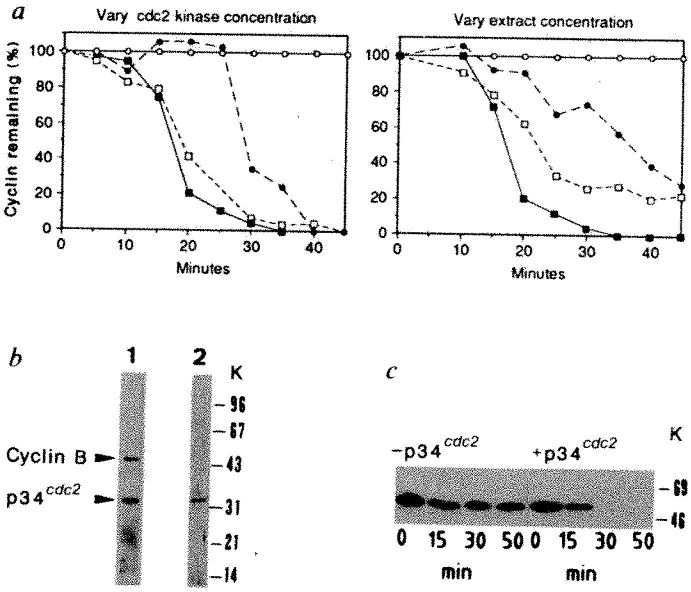

FIG. 1 Cyclin degradation in interphase extracts can be triggered by added cdc2 kinase. *a*, Left panel—effect of the level of cdc2 kinase activity on cyclin degradation. Kinase (prepared from starfish oocytes[6]) was added to give the following units of activity in the extracts: less than 6 U μl^{-1} (○); 12 U μl^{-1} (●); 24 U μl^{-1} (□); 48 U μl^{-1} (■). The level of cyclin is expressed as a fraction of the amount present at each time point without added kinase. Right panel—effect of dilution of the *Xenopus* extract on cyclin degradation. The starting extract (32 mg per ml protein) was the same as shown in the left panel. The extract was diluted twofold (□), fourfold (●) or 10-fold (○). For each time-point, the remaining cyclin is expressed as a fraction of the cyclin level in a parallel reaction with acetate buffer. All reactions contained 24 U μl^{-1} cdc2 kinase, 1 mM Mg^{2+}-ATP, 10 mM creatine kinase and 80 $\mu g\,ml^{-1}$ creatine phosphate. *b*, Silver-stained SDS-PAGE analysis of the two cdc2 kinase preparations. Lane 1, p34^{cdc2} associated with cyclin B (prepared as in ref. 16). Lane 2, homogeneous p34^{cdc2} catalytic subunit (prepared as in refs 18 and 28). Relative molecular mass (M_r) markers shown on the right ($M_r \times 10^{-3}$). *c*, Cyclin degradation induced by the addition of the p34^{cdc2} catalytic subunit. The cdc2 kinase shown in *b*, lane 2 (20 U μl^{-1}) was added to the *Xenopus* extract together with ^{35}S-labelled cyclin. Cyclin degradation was monitored by SDS-PAGE and autoradiography. M_r markers, right ($M_r \times 10^{-3}$).

METHODS. *Xenopus* eggs were activated by electric shock and extracts prepared 40 min later by centrifugation at 100,000*g* for 1 h as previously described[15]. The 100,000*g* supernatants were frozen in liquid nitrogen. A full-length complementary DNA clone of sea urchin (*Arbacia punctulata*) cyclin was transcribed and the RNA translated in reticulocyte lysate in the presence of 7.5 $\mu Ci\,\mu l^{-1}$ [^{35}S]methionine as described previously[16]. The [^{35}S]cyclin and cdc2 kinases were added in less than 10% of the final incubation volume. One unit of kinase activity is defined as 1 pmol phosphate transferred from ATP to histone H1 per min. Cycloheximide (10 $\mu g\,ml^{-1}$) was added to block further protein synthesis. At the indicated times of incubation at 22 °C, samples were diluted sixfold into SDS-gel sample buffer and analysed on 10% polyacrylamide gels. The gels were exposed to preflashed X-AR film (Kodak) and the cyclin bands quantified on an LKB ultroscan XL laser densitometer. To measure cdc2 kinase activity in the reactions, samples were diluted 26-fold into 'extraction buffer' (EB, 80 mM β-glycerophosphate, 15 mM $MgCl_2$, 20 mM EGTA, 1 mM DTT, pH 7.3) and assayed for H1 kinase activity with calf thymus histones[28].

We conclude that cdc2 kinase promotes the destruction of cyclin by initiating a cascade of reactions that begins with protein phosphorylation and ends with proteolysis. The cascade, which may contain several steps, seems to have a built-in time delay corresponding roughly to the normal length of mitosis. The protein kinase activity of cdc2 kinase presumably triggers the cascade; we do not intend to imply that cdc2 kinase contains a cryptic protease, or even that the putative protease is directly activated by cdc2-mediated phosphorylation.

To test the idea that the kinase activity of the added p34^{cdc2} triggered proteolysis, we added 6-dimethylaminopurine (DMAP) to the reactions. This ATP analogue inhibits the histone H1 kinase activity of cdc2 kinase[15,20]. Figure 2*a* shows that 1 mM DMAP substantially, although not completely, inhibited cyclin destruction; it also partially protected cdc2 kinase from inactivation. The activity of the kinase can still be measured under these conditions because the DMAP is diluted below its active concentration in the histone kinase assay. This interpretation is subject to the reservation that DMAP is an adenine analogue and might inhibit ATP-dependent reactions other than protein phosphorylation. To strengthen the case, we tested a more specific inhibitor of cdc2 kinase, the histone H1-like peptide KTPKKAKKPKTPKKAKKL (single-letter amino-acid code; KTPK peptide) which contains a repeat of the phosphorylation site for growth-associated H1 kinase[21] (a homologue of the mitotic cdc2 kinase[22]). This peptide is an excellent substrate for starfish cdc2 kinase and competitively inhibits the phosphorylation of histone H1 by this enzyme. At a peptide concen-

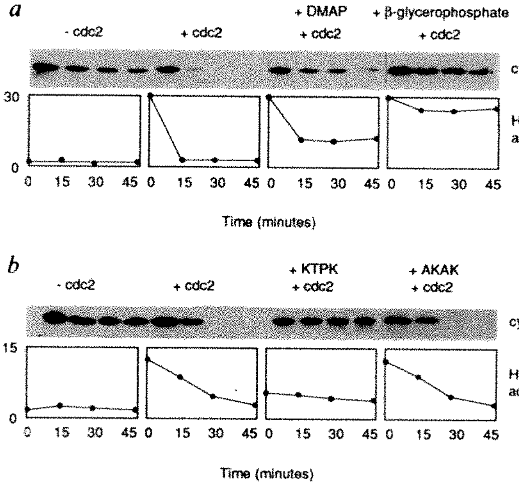

FIG. 2 Cyclin degradation is inhibited by 6-DMAP, β-glycerophosphate (β-gly) and KTPK peptide. The experiment was performed as described in Fig. 1. *a*, 1 mM DMAP or 20 mM magnesium-β-glycerophosphate were added to the extract together with the cdc2 kinase (30 U μl^{-1} final concentration as measured in the extract at zero time); *b*, Synthetic H1 peptide KTPKKAKKPKTPKKAKKL[21] (0.25 mM) or the control peptide AKAKKTPKAKK (0.6 mM) were added, together with 12 U μl^{-1} cdc2 kinase. The histone H1 kinase activity (in units μl^{-1}) measured at time zero in the presence of KTPK peptide was reduced because this peptide partially inhibited the kinase activity of cdc2 even after dilution in the assay.

tration of 100 μM in assay buffer (that is, without egg extract), the phosphorylation of 1 mg ml^{-1} histone H1 by 5 U μl^{-1} of cdc2 kinase was inhibited by 80%. At 250 μM, we obtained 95% inhibition. In the concentrated egg extracts, 250 μM peptide inhibited the incorporation of ^{32}P into specific proteins catalysed by 15 U μl^{-1} of added cdc2 kinase, so that the pattern of protein phosphorylation in the extract looked like an interphase pattern[15]. The peptide concentration required to inhibit cdc2 kinase-dependent phosphorylation in the extracts seems high, as endogenous substrates for the kinase are probably present at much lower concentrations than 250 μM. But the extracts are very concentrated and the peptide may absorb to negatively charged components such as RNA and is probably degraded fairly rapidly. We found that 250 μM was the lowest concentration at which the peptide inhibited cdc2 kinase-induced cyclin degradation (Fig. 2*b*). As a control, we used a shorter peptide with a similar amino-acid composition: AKAKKTPKAKK. This peptide did not inhibit the H1 kinase activity of purified cdc2 kinase, did not inhibit cdc2 kinase-dependent phosphorylation in the crude extract and, even at 600 μM, did not prevent cdc2 kinase-induced cyclin degradation (Fig. 2*b*). We conclude that the protein kinase activity of p34^{cdc2} is essential for inducing cyclin degradation.

Certain compounds such as β-glycerophosphate salts stabilize MPF and histone H1 kinase activities from frog eggs[17,23]. This might reflect the stabilization of cyclin against degradation. To test this idea we added 20 mM magnesium-β-glycerophosphate to the *in vitro* destruction assay. Figure 2*a* shows that this completely stabilized cyclin against degradation triggered by added cdc2 kinase. Moreover, the cdc2 kinase activity remained high in the presence of the added β-glycerophosphate. Although Erikson and Maller recently showed that β-glycerophosphate partially inhibited the kinase activity of purified p34^{cdc2} (ref. 24), this does not seem to be the case in crude extracts and we routinely assay histone kinase in 80 mM β-glycerophosphate; in fact, omitting it from the assay buffer tended to decrease the activity. Paradoxically, therefore, although the destruction of cyclin is initiated by a protein kinase, it could involve a protein phosphatase, as sodium-β-glycerophosphate is thought to act as a phosphatase inhibitor. Nevertheless, okadaic acid, a powerful inhibitor of phosphatases[25] type-1 and type-2A, did not inhibit the destruction of cyclin induced by cdc2 kinase in our system; if anything, it accelerated cyclin destruction[26]. Thus if β-glycerophosphate inhibits cyclin degradation by inhibiting a phosphatase, the phosphatase cannot be of type-1 or type-2A, and it presumably acts downstream of cdc2 kinase in the pathway leading to cyclin proteolysis. Irrespective of the precise mechanism, these data suggest that β-glycerophosphate salts help stabilize MPF by preventing cyclin degradation.

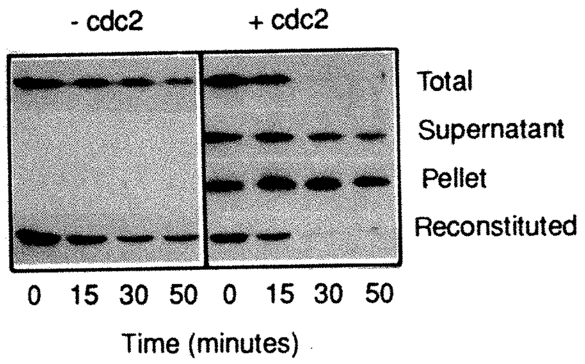

FIG. 3 cdc2 kinase-induced degradation of cyclin requires high M_r components. Cyclin was incubated in the complete reaction mix described in Fig. 1 (total), or with 150,000*g* supernatant depleted of high M_r material (supernatant), or with the pellet from this spin resuspended in acetate buffer (pellet), or a mixture of both (reconstituted), without (left panel) or with (right panel) 20 U μl^{-1} cdc2 kinase.

We previously found that 'particulate material' that could be sedimented only in very high centrifugal fields was involved in the activation of cdc2 kinase[15]. As a first attempt to fractionate the cyclin destruction machinery we tested whether high-speed supernatants could support cyclin destruction. By themselves, neither the high-speed supernatant nor the pellet were capable of destroying cyclin when cdc2 kinase was added (Fig. 3). When the pellet and the supernatant were mixed to reconstitute the extract, however, destruction was restored. This suggests that at least two components besides cyclin and cdc2 are required for cyclin destruction, one of which is included in the particulate material.

A threshold concentration of cyclin is required to activate cdc2 kinase[27], probably by permitting or promoting post-translational modifications of p34^{cdc2}. The results presented here show that when cdc2 kinase activity exceeds a certain level, it triggers a series of reactions that lead to the proteolysis of cyclin. This is evidence for the existence of a negative feedback loop that could form the basis of a minimum cell-cycle oscillator, as shown in Fig. 4. The system cannot reach equilibrium; it keeps oscillating, because threshold levels of both cyclin protein and cdc2 kinase activities trigger post-translational reactions with built-in time delays, and the destruction of cyclin causes the loss of cdc2 kinase activity. The period of the oscillator is determined in part by the rate of cyclin synthesis and in part by the time constants of the slow reactions, with cyclin degradation as the ultimate irreversible step. Figure 4 describes a possible mechanism by which cdc2 kinase could trigger cyclin degradation. In this model, mitotic cdc2 kinase increases the phosphorylation of a protein, X, which in turn activates cyclin degradation. At the end of M phase, after cyclin has been destroyed, cdc2 kinase is inactivated and so is the proteolysis machinery. The termination of cyclin proteolysis is an important feature of the system, for otherwise the next cycle could never take place. An alternative model, not illustrated here, is suggested by the observation that cyclin itself is phosphorylated during mitosis, probably by cdc2 kinase[5,7]. According to this model, alterations to cyclin

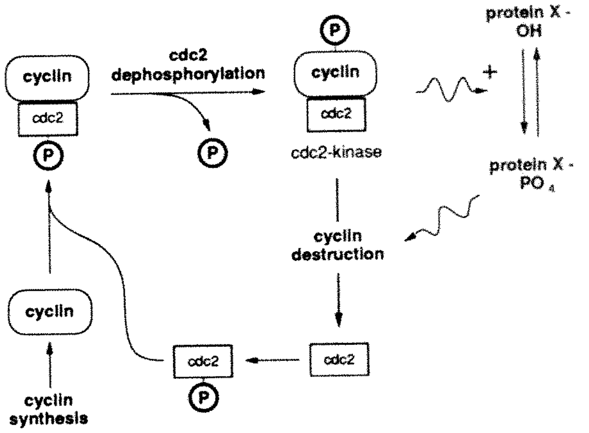

FIG. 4 A model to explain the oscillation of cdc2 kinase in early *Xenopus* embryos. The phosphorylated cdc2–cyclin complex shown at top left has no cdc2 kinase activity. It is activated by dephosphorylation of its p34^{cdc2} subunit. One of the cellular targets for cdc2 kinase is the hypothetical protein X. The phosphorylated form of protein X activates the protease that destroys cyclin. Once cyclin has been destroyed, cdc2 kinase is rapidly inactivated, either by rephosphorylation of the p34^{cdc2} subunit as shown here, or by combination with an inhibitor. The new synthesis of cyclin eventually permits reactivation of cdc2 kinase, presumably by promoting dephosphorylation of the p34^{cdc2} subunit. Cell-cycle oscillations in cdc2 kinase result from the continual accumulation of cyclin followed by its destruction, which is triggered by the active form of cdc2 kinase.

itself rather than to the protease would regulate the destruction of cyclin. Further work is necessary to distinguish between these possibilities. ☐

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New variation on the translocation of proteins during early biogenesis of apolipoprotein B

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APOLIPOPROTEIN B (apo B) is crucial for the transport of cholesterol in humans. It is a large secretory protein that mediates the uptake of low-density lipoproteins and renders several forms of lipid droplets soluble in the blood[1–3]. The binding of lipid by apo B also prevents this hydrophobic protein from precipitating in aqueous solution[4]. In the endoplasmic reticulum, nascent secretory proteins must be translocated through an aqueous channel in the membrane into the aqueous lumen[5], so some novel form of processing may be necessary to maintain the solubility of apo B during its translocation. We have discovered that the biogenesis of apo B in cell-free systems does indeed involve a new variation on protein translocation: unlike typical secretory proteins, apo B is synthesized as a series of transmembrane chains with large cytoplasmic domains and progressively longer amino-terminal regions that are protected against added proteases during the translocation process. In contrast to typical transmembrane proteins, these transmembrane chains are not integrated into the bilayer. Moreover, the transmembrane chains with the shortest protected domains are precursors of forms whose protection is progressively extended to cover the length of the protein. This stepwise conversion occurs post-translationally for the most part. We propose a model on the basis of these findings for the biogenesis of apo B.

Studying early events in the biogenesis of full-length apo B-100 (relative molecular mass of 550,000) is difficult *in vivo* because of the lack of synchrony in synthesis and degradation of most of the chains[6]; also the size of apo B-100 makes its synthesis *in vitro* technically daunting. Lengths of apo B shorter than B-100, however, are also secreted by cells. In humans, intestinal cells secrete apo B-48, which constitutes the N-terminal 48% of B-100, on chylomicrons[7,8]. In addition, a rare mutant species of apo B corresponding to the N-terminal 37% of B-100 (ref. 9) has been detected on lipoprotein particles in humans. Finally, lengths of apo B as short as the N-terminal 15% of apo B-100 are secreted from heterologous transfected hepatocytes (unpublished observation). Therefore, if any unusual translocational steps are involved in the synthesis of apo B, they are likely to occur during the synthesis of an amino-terminal domain of apo B. We therefore chose to study the N-terminal 15% of apo B-100 (termed apo B-15) in cell-free systems. Apo B-15 includes an N-terminal cleaved signal sequence of 27 amino acids and several hydrophobic regions that are representative of those occurring throughout apo B-100 (refs 10, 11) and which are assumed to bind to lipids. But these hydrophobic regions contain no more than 13 consecutive hydrophobic amino acids, and none constitutes a membrane-spanning domain[10].

When synthesized in the presence of microsomal vesicles, apo B-15 is translocated across the membranes, as evidenced by glycosylation (Fig. 1*a*). After digestion with proteinase K, the majority of translocated chains are cleaved, yielding several discrete bands that are suggestive of a family of transmembrane proteins with different lengths protected against digestion (Fig. 1*b*, lane 4). Proteolysis with trypsin and proteinase K together did not change this pattern of bands (data not shown). Immunoprecipitation of the protease-digested translation products with antipeptide sera directed against amino-acid residues 12–27 of mature apo B showed that these bands represent different N-terminal lengths of apo B (Fig. 1*c*). These N-terminal lengths are protected by virtue of the translocation process, rather than any intrinsic resistance to protease or nonspecific interaction with the membrane, because apo B-15 is completely digested (1) in the absence of vesicles; (2) in the presence of vesicles solubilized with detergent; (3) in the presence of membranes rendered incompetent for translocation by pretreatment with *N*-ethyl maleimide (Fig. 1*b*), or (4) in the presence of membranes depleted of the signal recognition particle (data not shown). These results strongly suggest that the amino-terminal region of apo B is synthesized as a transmembrane protein, in contrast to conventional secretory proteins.

Unlike typical integral membrane proteins, however, these transmembrane forms of apo B are not integrated into the bilayer. An aliquot of apo B-15 synthesized in the presence of microsomal vesicles (under conditions identical to those used for Fig. 1*b*) was mixed with aliquots of the simple secretory protein, bovine prolactin, and an integral transmembrane protein called S·gG·ST·P (a chimaeric protein that includes the IgM stop-transfer sequence[12]), each of which was translated *in vitro* independently in the presence of microsomal membranes. The pooled sample was divided in half and treated with either Tris-buffered sucrose (pH 7.5) or 0.1 M sodium carbonate (pH 11.5) before centrifugation to distinguish integral from peripheral proteins[13]. As expected, all three proteins were found in the pelleted membranes when treated with Tris-buffered sucrose, the simple secretory protein was extracted by carbonate, whereas the integral transmembrane protein was retained in the bilayer. Apo B-15, however, was extracted from the membrane in amounts comparable to that of the simple secretory protein (Fig. 2).

Finally, we used pulse-chase analysis to show that the various transmembrane forms were progressive intermediates in the formation of fully protected apo B-15. First, we found that 16–18 min are necessary for a chain of apo B-15 to be completely synthesized in the wheat-germ translation system supplemented with membranes (data not shown). Translation of apo B-15 was then initiated in the presence of membranes and [^{35}S]cysteine, and after 10 min labelling, a 1,000-fold excess of unlabelled cysteine was added. As shown in Fig. 3, the transmembrane form with the shortest protected domain predominates early in the incubation, then progressively decreases. Conversely, the fully protected chains accumulate steadily, reaching a maximum late in the incubation. The forms having intermediate lengths protected are maximal mid-way through the incubation. We conclude that this progressive conversion to the fully protected forms occurs mainly post-translationally, because synthesis of the last chains labelled in our 10-min pulse should be complete by 30 min.

Our results suggest a model for the biogenesis of apo B-15 (Fig. 4). First, nascent apo B-15 is targeted to the membrane of the endoplasmic reticulum (ER)[14,15] and a short length of the nascent chain (a) is translocated into the lumen (step 1). Second, translocation of the domain immediately downstream of the first transmembrane region (domain b, Fig. 4) stops while elongation proceeds. The growing chain might cross the membrane at one or several discrete points (c, e in step 2). Third, translation is completed (a-f, step 3). In the figure, the ribosome is released, but subsequent events may depend on its attachment. At this point, added proteases will digest the cytoplasmic domains, leaving only the shortest translocated domain (and other tiny luminal fragments if the chain crosses the bilayer at more than one point, as in region c in step 3). After translation, successive domains become protected from proteases: at this stage, protease digestion reveals that several N-terminal fragments are protected which become progressively longer (step 4). This stepwise conversion may be due to progressive translocation of C-terminal domains into the lumen (as shown in step 4), or it may result from conformational changes in the transmembrane chains that render protease-susceptible sites inaccessible. Lastly, the chain becomes fully protected, either as a result of complete translocation into the lumen (step 5, top) (the chain may remain associated with the inner leaflet of the bilayer), or because it acquires a transmembrane conformation that makes the entire chain resistant to proteases (step 5, bottom).

Previously, nascent apo B was assumed to be translocated completely across the ER membrane co-translationally, whereupon it became associated with the inner leaflet rather than spanning the bilayer[1,16]. This hypothesis stemmed from

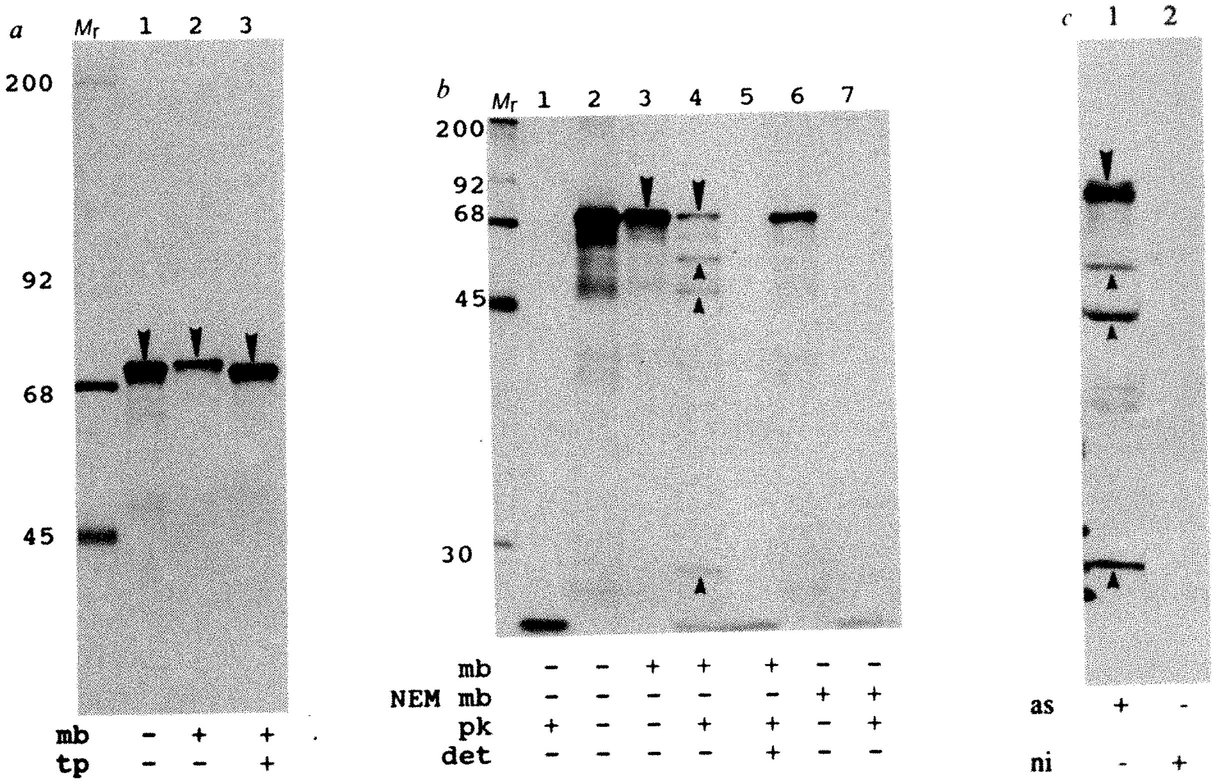


FIG. 1 *a*, Expression of Apo B-15 *in vitro*. Apo B-15 was translated in the absence (lane 1), or presence (lanes 2 and 3) of microsomal membranes (mb). In lane 3, a tripeptide (Asn-Tyr-Thr) competitor for *N*-linked glycosylation (tp) was added at the start of translation. Downward-pointing arrows indicate the principal products of each reaction displayed by SDS-PAGE on 5-12% gradient gel. Molecular weight markers (M_r) from 45,000-200,000 (45-200K) are shown in the left lane. *b*, Proteolysis studies of Apo B-15. Apo B-15 was translated for 90 min in the absence (lanes 1 and 2) or presence of microsomal membranes (mb; lanes 3-5), or in the presence of vesicles pretreated with *N*-ethyl maleimide (NEM mb; lanes 6 and 7). After translation, the samples were incubated on ice for 30 min either alone (lanes 2, 3 and 6), with 0.4 mg ml^{-1} proteinase K (pk; lanes 1, 3 and 7), or with proteinase K and 0.5% Triton X-100 (det; lane 5) before boiling in SDS. Aliquots were then displayed on a 12-17% gradient gel by SDS-PAGE and autoradiography. In lane 4, the downward-pointing arrow indicates the fraction of chains that are fully protected from digestion by proteinase K (compare with the band indicated by the arrow in lane 3). The upward-pointing arrows indicate the principal fragments that are partially protected from digestion by proteinase K. Molecular weight markers (M_r) from 30-200K are shown in the left lane. *c*, Immunoprecipitation of the fragments of apo B-15 partially protected from digestion by proteinase K. Lane 1, Immunoprecipitation with antipeptide sera (as) to amino acids 12-27 of apo B-100. The upward-pointing arrows indicate the principal transmembrane fragments protected from digestion by proteinase K. The downward-pointing arrow indicates full length apo B-15. Lane 2, Non-immune serum (ni).

METHODS. The complementary DNA encoding the N-terminal 15% of apo B-100 (ref. 10) followed by a termination codon (TAG), was engineered downstream of the SP6 promoter and expressed by transcription-linked[24] translation in a wheat germ cell-free system for 90 min in the presence or absence of vesicles derived from the ER of canine pancreas[23,24]. Aliquots of the [^{35}S]methionine-labelled products of translation were digested with proteinase K with or without nonionic detergents to solubilize the vesicles[25]. After boiling in sodium dodecyl sulphate (SDS), the samples were analysed by polyacrylamide gel electrophoresis in SDS (SDS-PAGE) followed by autoradiography.

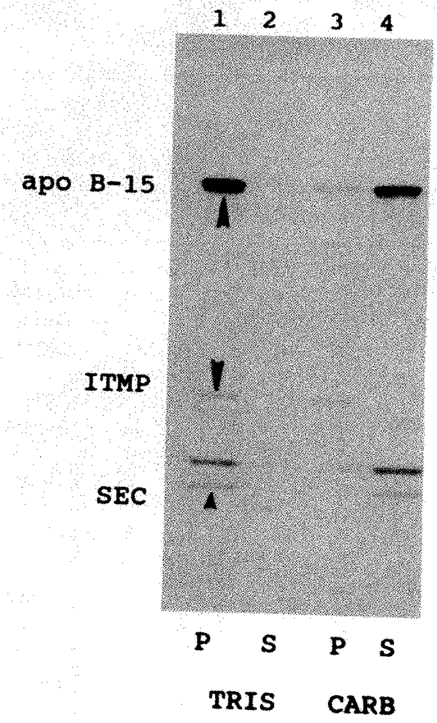
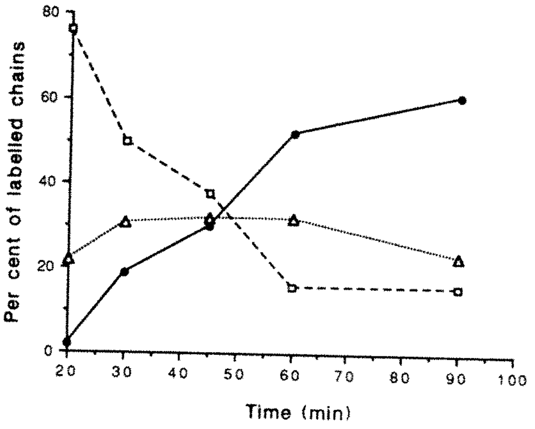


FIG. 2 Carbonate extraction of apo B-15. The simple secretory protein bovin prolactin (SEC), the glycosylated integral transmembrane protein (ITMP S·gG·ST·P (see text), and apo B-15 were synthesized independently *in vitr* in the presence of microsomal membranes and then mixed. The poole sample was divided in half and diluted 100-fold with either Tris-buffere 0.25 M sucrose, pH 7.5 (TRIS), or 0.1 M sodium carbonate, pH 11.5 (CARB and incubated on ice for 30 min. After centrifugation at 50,000 r.p.m. in Beckman-Ti-70.1 rotor at 4°C for 2 h, proteins in the supernates (S) wer precipitated with 15% trichloroacetic acid, washed with ethanol/ether, the dissolved in SDS; pellets (P) were also dissolved in SDS. Aliquots were the displayed by SDS-PAGE on a 5–12% gradient gel followed by autoradiography. The processed form of prolactin (SEC) is indicated by the small upward-pointing arrow, the glycosylated form of S·gG·ST·P (ITMP) by the downward-pointing arrow, and apo B-15 by the large upward-pointing arrow.

FIG. 3 Pulse-chase analysis of apo B-15. Apo B-15 was translated *in vitro* in the presence of microsomal vesicles and [^{35}S]cysteine. At 10 min, a 1,000-fold excess of unlabelled cysteine was added. (The effectiveness of the 1,000-fold dilution was demonstrated in a separate experiment in which either unlabelled cysteine or water was added to two similar ongoing translations of apo B-15, followed 10 min later by the addition of prolactin transcript. Densitometry revealed that the synthesis of radiolabelled prolactin was reduced by > 95% after dilution with unlabelled cysteine compared with water; data not shown.) Samples were removed at 20, 30, 45, 60 and 90 min for analysis by proteolysis followed by SDS-PAGE and autoradiography. The proportion of chains in each transmembrane band was quantitated by densitometry corrected for the distribution of cysteines. The values for the smallest band (squares, shortest), all other transmembrane bands (triangles, intermediate length), and the band corresponding to fully protected chains (filled circles) are plotted.



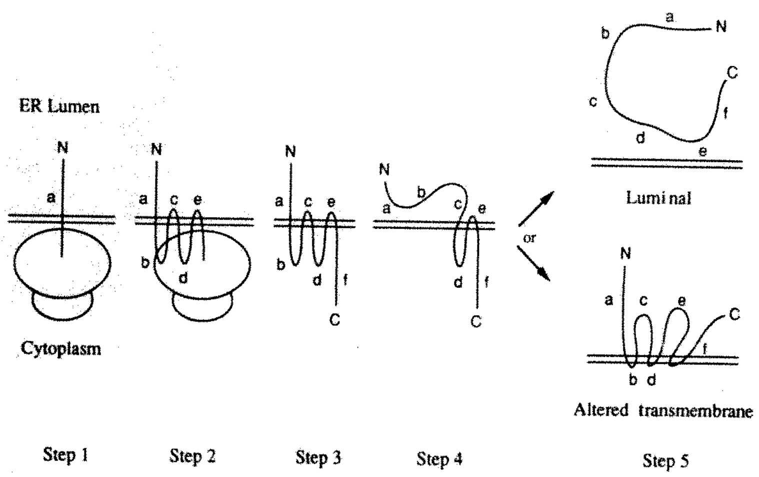


FIG. 4 Model of the biogenesis of apo B-15 (see text for details). N and C denote the amino and carboxy termini of the protein.

observations that apo B contains no more than 13 consecutive hydrophobic amino acids[10,11]—fewer than the minimum considered necessary to span a bilayer—yet nascent apo B is associated with the membrane of the ER[17,18]. But our results indicate that during its biogenesis, apo B-15 is a transmembrane protein with unusual features, suggesting that the N-terminal 15% of nascent apo B-100 might also span the bilayer without being integrated into it.

The assembly of apo B containing lipoproteins may begin within the membrane of the ER[16,17]. It is tempting to speculate that the stepwise, post-translational lengthening of the protease-resistant N-terminal domain of apo B described here is necessary for the progressive assembly of the protein with lipid from the ER membrane. Thus the translocation of some proteins may be coupled to their assembly: the membrane of the ER may participate in the assembly of apo B and other complex proteins or particles, rather than acting simply as a barrier between intracellular compartments. Alternatively, the delay in apo B translocation may reflect the unfavourable kinetics of translocating such a hydrophobic secretory protein.

These unusual translocational steps may be involved in the regulation of the biogenesis of apo B. Control of apo B secretion occurs after transcription[19] and probably after translation, because most of the apo B synthesized seems to be degraded in cultured hepatocytes[6]. Chains of apo B that do not complete the multistep process of translocation described here may remain in the membrane, destined for a degradative[20,21] rather than a secretory pathway.

Transmembrane proteins are usually integrated in the membrane by the action of stop-transfer sequences, but proteolysis and carbonate extraction of apo B-15 indicates that stop-transfer and integration might be dissociated. Perhaps discrete topogenic sequences will be identified that mediate each of these normally coupled events in the biogenesis of transmembrane proteins. Thus, apo B could contain sequences that direct stop-transfer but which have insufficient consecutive hydrophobic amino acids to mediate integration[14].

The relationship between the transmembrane chains and the translocational machinery[22] is unknown. These intermediates span the membrane without being integrated into it, and they ultimately appear fully protected and probably completely translocated. Thus, the transmembrane intermediates may still be associated with translocational machinery, in a state in which translocation is arrested but may subsequently proceed. ☐

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Widespread distribution and fitness contribution of *Xanthomonas campestris* avirulence gene *avrBs2*

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DISEASE-resistance genes introduced into cultivated plants are often rendered ineffective by the ability of pathogen populations to overcome host resistance[1–3]. The bacterial pathogen *Xanthomonas campestris* pathovar *vesicatoria* causes bacterial spot disease of tomato and pepper, and this pathogen has been shown to overcome disease resistance in pepper (*Capsicum annuum*) by evading the recognition and defence response of the host plant[4–6]. Numerous resistance genes to bacterial spot have been identified in pepper and its wild relatives, each providing resistance to specific races of *X. c. vesicatoria*[7–13]. The resistance gene *Bs1*, for example, provides resistance to *X. c. vesicatoria* strains expressing the avirulence gene *avrBs1* (ref. 11); *Bs2* provides resistance to strains expressing *avrBs2* (ref. 13) and so on. We now report that *avrBs2* is highly conserved among strains of *X. c. vesicatoria*, and among many other pathovars of *X. campestris*. Furthermore, we find that *avrBs2* is in fact needed for full virulence of the pathogen on susceptible hosts. This implies that plants carrying *Bs2* can recognize an essential gene of the bacterial pathogen, which may explain why *Bs2* confers the only effective field resistance to *X. c. vesicatoria* in pepper.

In contrast to other *X. c. vesicatoria* avirulence genes which have been cloned[13], the widespread distribution of *avrBs2* is striking. Each of the other identified *X. c. vesicatoria* avirulence genes has a restricted distribution among the physiological races of the bacterial species[11–13], but *avrBs2* is found in all strains of the tomato and pepper races of *X. c. vesicatoria* tested, including the 20 representative strains we commonly use (data not shown) and over 500 other strains (R. E. Stall, personal communication).

The distribution of *avrBs2* extends to other pathovars of *X. campestris* that do not cause disease on pepper. When inoculated on pepper, *X. campestris* pathovars other than *X. c. vesicatoria* generally induce a hypersensitive defence response[14] on both pepper cultivars ECW (lacking *Bs2*) and ECW20R (homozygous for *Bs2*). As each resistance gene-avirulence gene interaction in pepper causes a phenotypically unique hypersensitive response[13,15], the *avrBs2* activity of each pathovar could be tested. The typical *avrBs2-Bs2* hypersensitive response is a light brown confluent necrosis that appears 12–24 h after inoculation

TABLE 1 Distribution of *avrBs2* activity and DNA homology within *Xanthomonas*

Strain	Hypersensitivity on ECW20R	*avrBs2* Homology
X. c. vesicatoria	yes	yes
X. c. alfalfae	yes	yes
X. c. phaseoli	yes	yes
X. c. malvacearum	yes	yes
X. c. campestris	yes	yes
X. c. vitians	yes	yes
X. c. vignicola	yes	yes
X. c. glycines	no	yes
X. c. holcicola	no	yes
X. c. oryzae	—	yes
X. c. citri	—	yes
X. fragariae	no	no

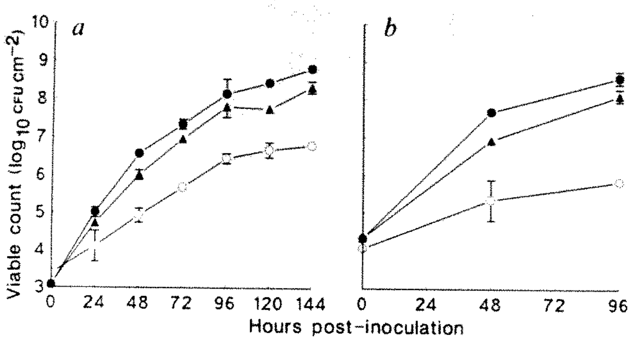

FIG. 1 *a*, Growth of *X. c. vesicatoria* strain 82-8 (●) and the strain with a spontaneous mutation at *avrBs2* (○) in the susceptible pepper cultivar ECW. The mutant strain displayed reduced virulence in the plant when compared to the wildptype; 5×10^6 CFU cm^{-2} and 5×10^8 CFU cm^{-2}, respectively. Complementation of the mutant with a plasmid-borne copy of *avrBs2* allowed the growth of the mutant to approach the wild-type level (▲). *b*, Growth of *X. c. alfalfae* (●) and a mutant carrying a marker-exchange mutation in *avrBs2* (○) on the susceptible alfalfa cultivar Moapa 69. The mutation at *avrBs2* reduces the ability of *X. c. alfalfae* to multiply in the susceptible host. Growth of the *X. c. alfalfae* exchange mutant carrying *avrBs2* from *X. c. vesicatoria* (▲) nears the wild-type growth level, indicating that the *X. c. vesicatoria avrBs2* clone complements the virulence of the alfalfa pathogen back to its wild-type level.
METHODS. Mean log$_{10}$ CFU cm^{-2} for both growth curves was determined essentially as described in ref. 6. The standard error for data points without visible error bars is less than 0.10 log$_{10}$ CFU cm^{-2}.

on ECW20R plants and is unlike any of the hypersensitive responses controlled by other pepper genes[13]. The pathovars that induced *Bs2* hypersensitivity specifically on ECW20R were classified as having *avrBs2* activity. Strains of *X. c.* pathovars *alfalfae*, *malvacearum*, *vignicola*, *vitians*, *campestris* and *phaseoli* display *avrBs2* activity (Table 1).

Evidence of *avrBs2* conservation was confirmed by Southern analysis[16]. For each pathovar, DNA probed with the cloned *avrBs2* gene from *X. c. vesicatoria* under stringent conditions (65 °C, 0.1 × SSC buffer, 0.1% SDS) revealed a conserved 2.35 kilobase (kb) *Sph*I fragment (Table 1). The pathovars *X. c. holcicola* and *X. c. glycines* have no *avrBs2* activity but display DNA homology to *avrBs2* nevertheless. In these cases, it is not known whether the *avrBs2*-homologous sequences are inactive or whether *avrBs2* inhibitors are acting in *trans*.

Although inoculation and DNA hybridization studies strongly suggest *avrBs2* activity in the other pathovars, proof of conservation was provided by cloning the *avrBs2*-homologous sequences of two of the pathovars. Libraries of *X. c. alfalfae* and *X. c. phaseoli* were made in the vector pUC119 (ref. 17). Colony hybridization with the *X. c. vesicatoria avrBs2* gene identified homologous clones in each library. The cloned inserts were transferred into the wide host-range vector pLAFR3 (ref. 18) and conjugated into a *X. c. vesicatoria* strain containing a mutation at *avrBs2* that renders the strain virulent on ECW20R plants. In both cases, the cloned gene complemented the *avrBs2* mutation and restored avirulence on ECW20R plants. The ability of the *X. c. alfalfae* and *X. c. phaseoli* clones to complement the *X. c. vesicatoria avrBs2* mutation confirmed that gene activity as well as DNA homology is conserved among these pathovars.

Conservation of *avrBs2* among all *X. c. vesicatoria* strains implies that *avrBs2* plays an important part in the fitness of the pathogen, a hypothesis tested by comparing the growth of *X. c. vesicatoria* with either a wild-type or mutant *avrBs2* gene in the susceptible host ECW. The wild-type strain grew to $\sim5\times10^8$ colony-forming units (CFU) cm^{-2} in the plant after 4 days. In the same period of time, the mutant grew to 5×10^6 (Fig. 1*a*), roughly the same level of growth as that of the wild-type bacterium in the resistant host (data not shown). When the mutation was complemented by a plasmid-borne copy of *avrBs2*, the virulence of the mutant was restored to near wild-type levels (Fig. 1*a*), showing that full virulence depends on *avrBs2*. In repeated testing, the spontaneous mutant was not complemented to full wild-type growth levels (Fig. 1*a*), suggesting that additional mutations affecting virulence may be present in the strain.

Conservation of *avrBs2* among the other *X. campestris* pathovars implies a role for *avrBs2* in the fitness of these pathogens as well. To test the hypothesis, a marker-exchange mutation was made in the *avrBs2*-homologous sequence from *X. c. alfalfae*, and growth of the wild-type and mutant strains in a susceptible alfalfa cultivar determined (Fig. 1*b*). The results indicate that mutation of the *avrBs2*-homologous sequence reduces the virulence of the alfalfa pathogen on its susceptible host by over 100-fold. Complementation of the *X. c. alfalfae* mutation with the *X. c. vesicatoria* avirulence gene restored the alfalfa pathogen to its former growth level, confirming the importance of *avrBs2* to the fitness of *X. c. alfalfae*.

Just as mammalian immunity is most effective when directed against highly conserved epitopes important to the fitness of a pathogen, we believe *Bs2* resistance has yet to be overcome in the field because *Bs2*-containing plants recognize a highly conserved bacterial signal involved in virulence. Mutations undoubtedly occur at *avrBs2*, but the reduced ability of the mutants to colonize *Bs2* plants prevents disease development. It is possible that *Bs2* resistance may break down in the field due to secondary mutations that restore virulence to the *avrBs2* mutants. Although in limited laboratory testing these types of mutations have not been recovered (data not shown), long-term field testing is required to confirm the durability hypothesis. The results presented here suggest that it may be possible to predict the durability of resistance genes in the future by analysing the conservation and importance of the bacterial signals they recognize.

Conservation of *avrBs2* among other *X. campestris* pathovars also suggests solutions to severe disease problems in other plant species; there is no effective genetic resistance in commercial tomatoes to *X. c. vesicatoria*, in bean to *X. c. phaseoli*, in cabbage to *X. c. campestris*, or in citrus to *X. c. citri*. When the *Bs2* gene is cloned and transferred to these other plant species, it may provide stable genetic resistance to these other diseases as well. □

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Highly conserved core domain and unique N terminus with presumptive regulatory motifs in a human TATA factor (TFIID)

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THE factor TFIID is one of several general factors that are necessary and sufficient for transcription initiation by mammalian RNA polymerase II[1,2]. Stable interactions with the common TATA element[3,4] lead both to template commitment and to the assembly of the other general factors into a functional preinitiation complex[5,6]. Consistent with its key role in the promoter activation pathway, human TFIID also seems to be a target for some regulatory factors, as evidenced both by physical[3,7,8] and functional[9-12] studies of interactions between these components. The evolutionary conservation of functional properties[13,14] led to the purification and cloning of yeast TFIID[15-19], the identification of presumptive structural motifs[15,19], and direct structure–function studies[20]. Here we report the cloning of a complementary DNA encoding a functional human TFIID. This reveals an evolutionarily conserved core which corresponds precisely to the 180-residue DNA binding/activation domain determined[20] for yeast TFIID, a near absolute conservation of component structural motifs (direct repeats, central basic core/lysine repeat, and sigma homology), providing further support for their functional importance, and a unique N-terminal structure that suggests involvement in species-specific regulatory factor interactions.

Overlapping human TFIID cDNAs (Fig. 1) were cloned following polymerase chain reaction (PCR) amplification with minimally degenerate primers that were chosen from the yeast TFIID core domain previously shown to be necessary and sufficient for DNA binding[20] and to be conserved in *Schizosac-*

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1          CTGAGAAGGGTGTGCTGGAGATGCTCTAGGAAAAAATTGAATAGTGAGACGAGTTCCAGCGCAAGGGTTTCTGGT

76     TTGCCAAGAAGAAAGTGAACATC ATG GAT CAG AAC AAC AGC CTG CCA CCT TAC GCT CAG GGC TTG
1                               M   D   Q   N   N   S   L   P   P   Y   A   Q   G   L

141    GCC TCC CCT CAG GGT GCC ATG ACT CCC GGA ATC CCT ATC TTT AGT CCA ATG ATG CCT TAT
15      A   S   P   Q   G   A   M   T   P   G   I   P   I   F   S   P   M   M   P   Y

201    GGC ACT GGA CTG ACC CCA CAG CCT ATT CAG AAC ACC AAT AGT CTG TCT ATT TTG GAA GAG
35      G   T   G   L   T   P   Q   P   I   Q   N   T   N   S   L   S   I   L   E   E

261    CAA CAA AGG CAG CAG CAG CAA CAA CAA CAG CAG CAG CAG CAG CAG CAG CAG CAG CAA CAG
55      Q   Q   R   Q   Q   Q   Q   Q   Q   Q   Q   Q   Q   Q   Q   Q   Q   Q   Q   Q

321    CAA CAG CAG CAG CAG CAG CAG CAG CAG CAG CAG CAG CAG CAG CAG CAA CAG GCA GTG GCA
75      Q   Q   Q   Q   Q   Q   Q   Q   Q   Q   Q   Q   Q   Q   Q   Q   Q   A   V   A

381    GCT GCA GCC GTT CAG CAG TCA ACG TCC CAG CAG GCA ACA CAG GGA ACC TCA GGC CAG GCA
95      A   A   A   V   Q   Q   S   T   S   Q   Q   A   T   Q   G   T   S   G   Q   A

441    CCA CAG CTC TTC CAC TCA CAG ACT CTC ACA ACT GCA CCC TTG CCG GGC ACC ACT CCA CTG
115     P   Q   L   F   H   S   Q   T   L   T   T   A   P   L   P   G   T   T   P   L

501    TAT CCC TCC CCC ATG ACT CCC ATG ACC CCC ATC ACT CCT GCC ACG CCA GCT TCG GAG AGT
135     Y   P   S   P   M   T   P   M   T   P   I   T   P   A   T   P   A   S   E   S

561    TCT GGG ATT GTA CCG CAG CTG CAA AAT ATT GTA TCC ACA GTG AAT CTT GGT TGT AAA CTT
155     S   G   I   V   P   Q   L   Q   N   I   V   S   T   V   N   L   G   C   K   L

621    GAC CTA AAG ACC ATT GCA CTT CGT GCC CGA AAC GCC GAA TAT AAT CCC AAG CGG TTT GCT
175     D   L   K   T   I   A   L   R   A   R   N   A   E   Y   N   P   K   R   F   A

681    GCG GTA ATC ATG AGG ATA AGA GAG CCA CGA ACC ACG GCA CTG ATT TTC AGT TCT GGG AAA
195     A   V   I   M   R   I   R   E   P   R   T   T   A   L   I   F   S   S   G   K

741    ATG GTG TGC ACA GGA GCC AAG AGT GAA GAA CAG TCC AGA CTG GCA GCA AGA AAA TAT GCT
215     M   V   C   T   G   A   K   S   E   E   Q   S   R   L   A   A   R   K   Y   A

801    AGA GTT GTA CAG AAG TTG GGT TTT CCA GCT AAG TTC TTG GAC TTC AAG ATT CAG AAC ATG
235     R   V   V   Q   K   L   G   F   P   A   K   F   L   D   F   K   I   Q   N   M

861    GTG GGG AGC TGT GAT GTG AAG TTT CCT ATA AGG TTA GAA GGC CTT GTG CTC ACC CAC CAA
255     V   G   S   C   D   V   K   F   P   I   R   L   E   G   L   V   L   T   H   Q

921    CAA TTT AGT AGT TAT GAG CCA GAG TTA TTT CCT GGT TTA ATC TAC AGA ATG ATC AAA CCC
275     Q   F   S   S   Y   E   P   E   L   F   P   G   L   I   Y   R   M   I   K   P

981    AGA ATT GTT CTC CTT ATT TTT GTT TCT GGA AAA GTT GTA TTA ACA GGT GCT AAA GTC AGA
295     R   I   V   L   L   I   F   V   S   G   K   V   V   L   T   G   A   K   V   R

1041   GCA GAA ATT TAT GAA GCA TTT GAA AAC ATC TAC CCT ATT CTA AAG GGA TTC AGG AAG ACG
315     A   E   I   Y   E   A   F   E   N   I   Y   P   I   L   K   G   F   R   K   T

1101   ACG TAATGGCTCTCATGTACCCTTGCCTCCCCCACCCCCTTCTTTTTTTTTTTTTAAACAAATCAGTTTGTTTTGGTA
355     T   OCH

1179   CCTTTAAATGGTGGTGTTGTGAGAAGATGGATGTTGAGTTGCAGGGTGTGGCACCAGGTGATGCCCTTCTGTAAGTGCC

1258   CACCGCGGGATGCCGGGAAGGGGCATTATTTGTGCACTGAGAACACCGCGCACGTGACCTGTGAGTTGCTCATACCGTG

1337   CTGCTATCTGGGCAGCGCTGCCCATTTATTTATATGTAGATTTTAAACACTGCTGTTGACAAGTTGGTTTGAGGGAGAA

1416   AACTTTAAGTGTTAAAGCCACCTCTATAATTGATTGGACTTTTTAATTTTAATGTTTTTCCCCATGAACCACAGTTTTT

1495   ATATTTCTACCAGAAAAGTAAAAATCTTTTTTAAAAGTGTTGTTTTTCTAATTTATAACTCCTAGGGGTTATTTCTGTG

1574   CCAGACACATTCCACCTCTCCAGTATTGCAGGACAGAATATATGTGTTAATGAAAATGAATGGCTGTACATATTTTTTT

1653   CTTTCTTCAGAGTACTCTGTACAATAAATGCAGTTTATAAAAAAAAAAAAAAAAAAAA   1710
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FIG. 1 Nucleotide and predicted amino-acid sequence of human TFIID cDNA. Shown is the combined sequence of three independent overlapping cDNA clones. The PCR product (nucleotide positions 651–862) contained in each of these is underlined with a solid line and sequences encoded by the primers with dashed arrows. The 1,005-base pair (bp) open reading frame is defined by translation start and stop codons (boxed in the nucleotide sequence) and encodes a polypeptide containing the characteristic TFIID homology core domain (amino acids 155–335, boxed) as well as an N-terminal region unique to this human TFIID polypeptide. A putative polyadenylation signal near the 3′ end is underlined.

METHODS. A novel computer program was used to calculate the minimum degeneracy of oligonucleotides necessary to specify five amino-acid stretches within the previously mapped TFIID core domain[21]. These were used as primers to amplify DNA fragments by PCR from a Namalwa cell cDNA library[28]. Five candidate products were selected on the basis of expected size and cross-hybridization to yeast TFIID probes at standard low stringency conditions[21]. Sequencing of the subcloned fragments revealed one that encoded a polypeptide with a high sequence homology to amino acids 91–161 of the *S. cerevisiae* TFIID. This insert was used to screen 10^6 recombinants from the same library under standard high stringency conditions[21]. Three independent clones of 1,258, 1,254 and 1,232 bp were sequenced in both directions using the chain termination method. One contained a poly(A) tail 685 bp 3′ of the TFIID ORF stop codon, whereas another contained the full ORF as evidenced by a start codon (ATG) in the correct reading frame preceded by stop codons (boxed) in the same reading frame.

charomyces pombe TFIID[21]. The combined sequence contains a 335-residue open reading frame encoding a polypeptide of calculated relative molecular mass (M_r) 37,160. Southern blots with short RNA probes showed hybridization with several high M_r placental DNA fragments, indicative either of several homologous genes or of a complex intron-exon structure (as observed for *Schiz. pombe*[21] and *Arabidopsis*[22]), whereas RNase protection assays indicated a low abundance of uniform length transcripts (data not shown).

Several assays showed that the cloned cDNA encodes a functional TFIID with TATA-box-binding and basal-level-transcription activities. A polypeptide (p38) of apparent M_r 38,000 was generated from expression of the cDNA in bacteria (data not shown) or translation of cDNA-derived transcripts in a reticulocyte lysate (Fig. 2*a*). Polypeptide p38 bound specifically to the TATA element of the adenovirus ML promoter as shown by the different sensitivities of two p38-dependent complexes to oligonucleotides with normal or mutant TATA sequences (Fig. 2*b*). One of these complexes was indistinguishable from the complex formed with natural human TFIID, whereas the second had a faster mobility; this suggests that the translated p38 protein may not be structurally equivalent to natural TFIID and/or that p38 may interact with or be modified by factors in the lysate.

When analysed in a cell-free transcription system reconstituted with general factors from HeLa cells, both the lysate-derived (Fig. 2*c*) and the bacterially expressed (Fig. 2*d*) human p38, as well as bacterially expressed yeast TFIID (Fig. 2*d*), could mediate basal level transcription as effectively as natural human TFIID. In contrast, although the stimulatory effect of the human transcriptional activator USF (which binds upstream of TATA on the ML promoter[3]) was readily apparent with native human TFIID, no detectable enhancement was observed with the bacterially expressed yeast or human TFIID polypeptides (Fig. 2*d*). Possibly related to this difference, the native human TFIID showed transcripts in the guanosine(G)-less cassette assay indicative of multiple initiations on the same template (Fig. 2*d* legend), whereas the bacterially expressed proteins did not.

Figure 3*b* details, and Fig. 4 summarizes schematically, a comparison of the human TFIID sequence with TFIID sequences from *Saccharomyces cerevisiae*, *Schiz. pombe*[21], *Arabidopsis thaliana*[22] and *Drosophila melanogaster* (M. Muhich, C. Iida and C. Parker, unpublished observations). There is a remarkable degree of sequence conservation (≥80% identity) and complete colinearity between the ~180-residue C-terminal regions of the factors. Moreover, this region correlates exactly with the region of *S. cerevisiae* TFIID previously shown[20] to be necessary and sufficient for TATA box binding and core promoter transcription. Altogether, these observations clearly argue for a conserved TFIID 'core' which explains the evolutionary conservation of TFIID function.

The TFIID core also contains the structural motifs (Fig. 3*b*, Fig. 4) noted previously[15,19] and in the accompanying paper[21]. The residues comprising these elements show an even more striking (near absolute) conservation from yeast through to humans, which argues strongly for a conservation of functional roles as follows. First, there are direct repeats[19] which allow an element of symmetry in the folded TFIID molecule. This has been proposed to be important for DNA binding, both by analogy to the dimeric structure of other site-specific DNA binding proteins and on the basis of mutational analysis[20] (T.Y.,

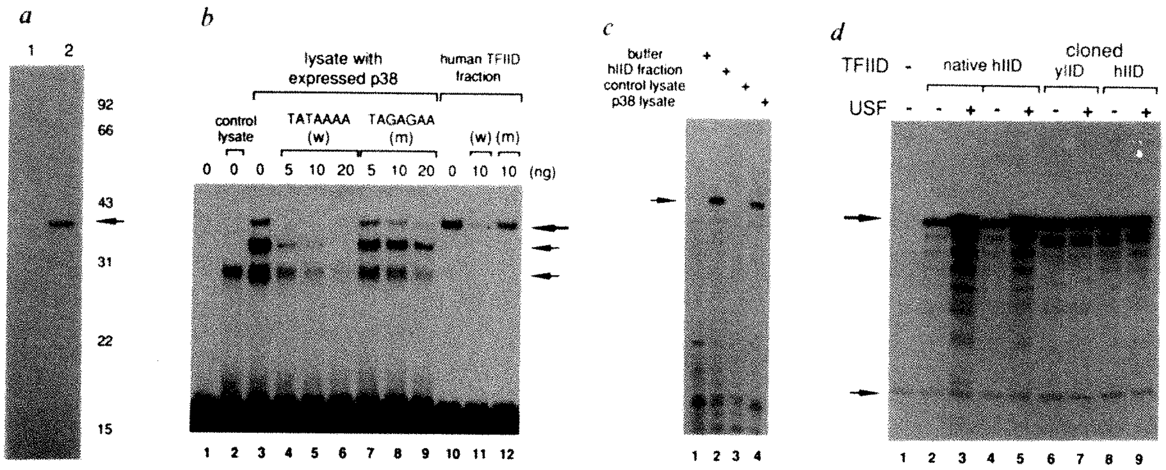

FIG. 2 *In vitro* expression and function of the human cDNA-encoded TFIID. *a*, SDS-PAGE analysis of [^{35}S]methionine-labelled TFIID produced in reticulocyte lysate. Lysates were programmed with no RNA (lane 1) or human TFIID cDNA-derived mRNA (lane 2). The size of the labelled TFIID polypeptide (arrow) based on size markers (not shown) is M_r 38,000. *b*, Site-specific binding of expressed and native human TFIID. Gel shift assays with a major late promoter (MLP) fragment contained: lane 1, no protein; lane 2, control lysate with no RNA; lanes 3–9, lysate programmed with human TFIID mRNA; lanes 10–12, amino-octyl fraction of native human TFIID[4]. Unlabelled oligonucleotides (MLP, positions −45 to −15) contained either a wild type (w) or a mutant (m) TATA box (as indicated) and were added as competitors in the nanogram amounts indicated above each lane. The bold arrow and the upper thin arrow indicate human TFIID specific complexes whereas the lower thin arrow indicates a nonspecific complex formed by a component in the lysate. *c*, Transcriptional activity of expressed human TFIID in a reconstituted system. Reactions containing an MLP template (pSmaF) and partially purified factors TFIIB, TFIIE/F and RNA polymerase II[29] were complemented with buffer (lane 1), DE52 fraction of human TFIID[4] (hIID) (lane 2), unprogrammed lysate (lane 3), and lysate programmed with human TFIID mRNA (lane 4). Specific transcripts measured by primer extension are indicated by the arrow. *d*, USF-stimulated transcription with human TFIID. Reactions containing an adenovirus MLP template with upstream sequences (−400 to +10) attached to the 380-bp G-less cassette pML(C$_2$AT)[29] and HeLa factors TFIIB, TFIIF/F, and RNA polymerase II were complemented with buffer (lane 1), DE52 fraction of native TFIID (lanes 2, 3), amino-octyl fraction of native TFIID (lanes 4, 5) and purified bacterially expressed yeast (lanes 6, 7) or human (lanes 8, 9) TFIID. A DE52 fraction of huamn activator USF[3] was present in lanes 3, 5, 7 and 9. Transcription from the G-free cassette was in the absence of GTP; the upper arrow indicates the full length cassette transcript resulting from specific initiation at +1 whereas the shorter ~30-bp spaced bands (lanes 2–5) are indicative of multiple initiations on the same template[29]. The lower arrow indicates the position of a labelled DNA fragment added as an internal standard.

METHODS. A human TFIID cDNA (containing nucleotides 1–1,258) in Bluescript SK was transcribed with T7 RNA polymerase and derived RNA was translated in rabbit reticulocyte lysates (Promega) after heat denaturation. Mobility shift assays[15] and primer extension[15] and G-free cassette[29] transcription assays were performed as previously described. *S. cerevisiae* and human TFIID cDNAs containing the coding regions were expressed in bacteria, and lysates were prepared as previously described[21]. The recombinant yeast (p27) and human (p38) TATA factors were purified to near homogeneity.

npublished results). Second, there is a sigma homology gion[15], which overlaps the C-terminal direct repeat and introuces an element of asymmetry into the direct repeat structure. his region of TFIID has been implicated in TATA box binding y deletion[20] and point mutagenesis studies (T.Y., unpublished sults) and we have suggested that it could provide the primary eterminants for the specificity of DNA binding. Third, there a repeat of basic residues[15] (notably lysines) in the central asic core connecting the direct repeats that has the potential r formation of an α-helix with basic residues on one face. Iutagenesis studies indicate that individual basic residues are equired neither for DNA binding nor for basal transcription T.Y., unpublished results), consistent with our earlier suggeson[15] that they might interact with certain types of activators. ourth, there are myc homologies, noted first by Gasch *et al.*[22] or the *Arabidopsis* and yeast proteins, between regions of TFIID nd the helix-loop-helix domains of a *myc*-related family of egulatory proteins. As discussed by these authors, this raises he possibility that these regions are important either for omotypic interactions within TFIID or for heterotypic interacions with distinct regulatory factors.

In striking contrast to the conserved C-terminal regions, the -terminal regions of TFIID differ markedly both in size and equence (Fig. 4). The human TFIID N terminus lacks the high ensity of charged residues previously noted[21] in yeast TFIID, ut contains obvious structural features that could be functionally related to similar structural motifs in other regulatory factors. These include (see Fig. 3*a*): a region containing 34 consecutive glutamines (the Q-run) flanked by two regions (STP-1 and STP-2) rich in serine (S), threonine (T) and proline (P) residues. The latter show a general similarity to Ser, Thr, (Pro)-rich regions in regulatory proteins like SP1[23] and GAL11[24], whereas the Ser-Pro, Thr-Pro and Tyr-Pro/Pro-Tyr repeats (usually preceded, followed or interspersed with an Ala, Ile, Leu or Met residue) are reminiscent of the reversibly phosphorylated heptad repeat (Tyr-Ser-Pro-Thr-Ser-Pro-Ser) in the largest subunit of RNA polymerase II[25]. Similarly the Q-run and adjacent Gln-rich regions resemble polyglutamine and Gln-rich regions present in other factors[23,24,26] and implicated, in some cases, as activation domains[23,27]. These regions might well serve as interfaces for intermolecular protein-protein interactions.

Yeast TFIID structure-function studies[20] and comparative sequence analyses (Fig. 3) suggest that the human TFIID N terminus is not essential for basal transcription functions. Unlike natural human TFIID[3,7,10-12], however, neither bacterially expressed yeast nor human TFIID seem capable of mediating the normal functions of certain activators (like USF) in reconstituted cell-free systems (Fig. 2*d*; M. Meisterernst, unpublished observations). This suggests that the human N terminus might be involved in mediating regulatory factor interactions, either with TFIID itself or with other general factors, and that these functions might be encoded in separate factors (such as GAL11)

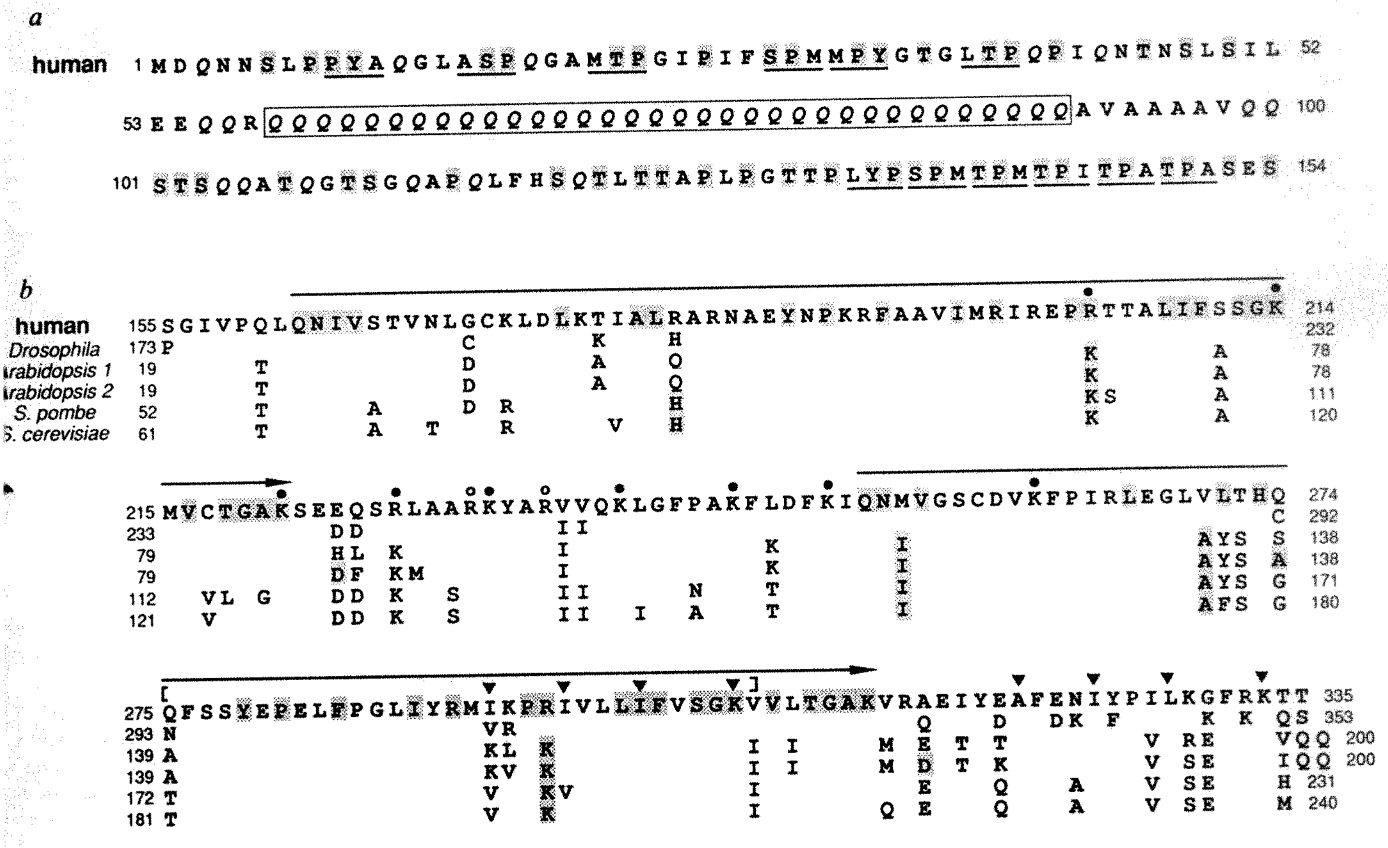
a
human
1 M D Q N N S L P P Y A Q G L A S P Q G A M T P G I P I F S P M M P Y G T G L T P Q P I Q N T N S L S I L 52
53 E E Q Q R Q Q Q Q Q Q Q Q Q Q Q Q Q Q Q Q Q Q Q Q Q Q Q Q Q Q Q Q Q Q Q Q Q Q A V A A A A V Q Q 100
101 S T S Q Q A T Q G T S G Q A P Q L F H S Q T L T T A P L P G T T P L Y P S P M T P M T P I T P A T P A S E S 154
b
human
Drosophila
rabidopsis 1
rabidopsis 2
S. pombe
S. cerevisiae
155 S G I V P Q L Q N I V S T V N L G C K L D L K T I A L R A R N A E Y N P K R F A A V I M R I R E P R T T A L I F S S G K 214
232
173 P
19
19
52
61
78
78
111
120
215 M V C T G A K S E E Q S R L A A R K Y A R V V Q K L G F P A K F L D F K I Q N M V G S C D V K F P I R L E G L V L T H Q 274
292
233
79
79
112
121
138
138
171
180
275 Q F S S Y E P E L F P G L I Y R M I K P R I V L L I F V S G K V V L T G A K V R A E I Y E A F E N I Y P I L K G F R K T T 335
293 N
139 A
139 A
172 T
181 T
Q S 353
V Q Q 200
I Q Q 200
H 231
M 240

FIG. 3 The human TATA factor consists of a highly conserved TFIID core domain and a unique N-terminal structure. *a*, The amino-acid sequence of the nonconserved N-terminal region of the human TATA factor (single-letter amino-acid code). The potentially interesting primary structure motifs highlighted include a glutamine repeat (boxed) which is flanked by two regions (STP-1 and 2) that are rich in serine, threonine, and proline and characterized by triplets (underlined) containing Ser-Pro, Thr-Pro, and Pro-Tyr/Tyr-Pro pairs preceded or followed by a Met, Ile, Leu, or Ala residue. Alternative arrangements of repeating triplets (such as Pro-Met-Thr, Pro-Ile-Thr) are also possible. *b*, The sequence of the human TFIID core domain is displayed in the top line, whereas the lower lines show only amino acids that differ in the TFIID sequences from other species. Putative structural domains that are discussed in the text are indicated as follows: (1) direct repeats, long arrows indicate regions containing the two interrupted repeats whose constituent residues are shaded; (2) basic repeat, solid circles (lysines) and open circles (arginines) indicate residues in the central basic core which lie on one face of potential α-helical structures; (3) sigma homology, the region with an overall sequence similarity to the sigma 2.3 and 2.4 domains is indicated by brackets while solid inverted triangles denote residues, in two small regions, that correspond to those most highly conserved in the 2.4 regions of various sigma factors. The regions with sequence similarities to the helix-loop-helix proteins (Gasch *et al.*[22]) extend from residues 201 to 220 in direct repeat 1 and from 269 to 314 in direct repeat 2.

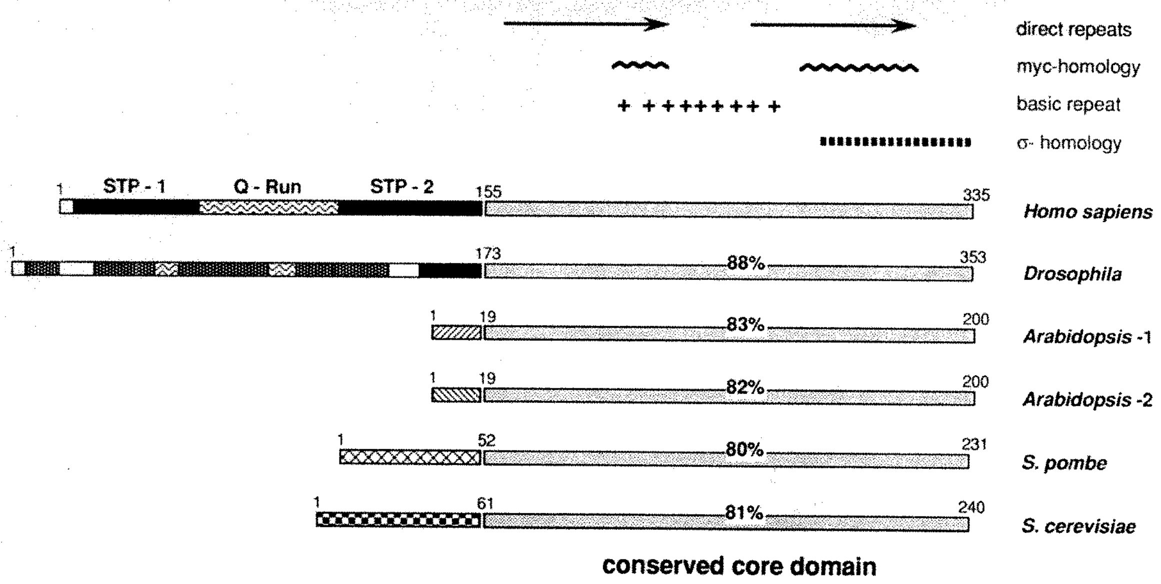

FIG. 4 Evolutionary conservation of the TATA factor primary structure. The degree of sequence identity (relative to human) within the conserved C-terminal core domains of the TFIIDs from human, *Drosophila* (M. Muhich, C. Iida, C. Parker, unpublished results), *Arabidopsis*[22], *Schiz. pombe*[21] and *S. cerevisiae*[15] are summarized. Also indicated are the positions in the core of the structural domains discussed in the text. The N-terminal regions of these proteins differ markedly both in length and amino-acid composition and sequence, except that the *Drosophila* N terminus shows some sequenc similarity (including Gln-rich and Ser, Thr, Pro-rich regions) with the huma N terminus. For the *Drosophila* N terminus the black and dark grey boxe represent, respectively, regions with strong and weaker sequence similaritie to the human STP regions, and short runs of glutamine (Q-runs) are indicate as for the human N terminus.

in lower eukaryotes whereas in mammalian cells they might require either modifications of the N-terminal domains or interactions with other cofactors. The latter situation is also suggested by size differences between natural human TFIID (M_r 120,000)[2] and the cloned TATA-binding protein (M_r 37,160). Although it is tempting to invoke the N-terminal domains in regulatory factor interactions, it is important to bear in mind the great differences in the N-terminal domains from various species and their near absence in *Arabidopsis* TFIID. Thus, in some cases regulatory factor functions may be mediated totally or in part through direct (or cofactor-dependent) interactions with the conserved TFIID core or with other general factors.

In summary, we have described the cloning and functional characterization of a human TATA factor (TFIID). Sequence comparisons with TFIID from other species suggest both structural similarities that account for conserved functions and dissimilarities that may reflect distinct regulatory mechanisms. ☐

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Arabidopsis thaliana contains two genes for TFIID

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THE general transcription initiation factor TFIID plays a primary part in the activation of eukaryotic genes transcribed by RNA polymerase II. Binding of TFIID to the TATA box initiates the assembly of other general transcription factors as well as RNA polymerase II at the promoter resulting in a preinitiation complex capable of accurate transcription initiation *in vitro*[1–3]. Human TFIID has been shown to interact with various regulatory factors[4–8]. The observation that stimulation of transcription by different *trans*-acting factors is mediated through distinct TATA elements led to the suggestion that different types of TFIID may exist in yeast[9–11], humans[12–15] and plants[16]. Here we report the cloning and characterization of two distinct TFIID complementary DNA clones from *Arabidopsis thaliana*. Furthermore, we have found that TFIID from *Arabidopsis* and other organisms shows homology to helix-loop-helix proteins.

Several genomic clones from *Arabidopsis thaliana*, all derived from the same locus, were isolated using the recently isolated yeast TFIID gene[17–21] as a probe. To obtain related cDNAs we

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G. 1 Nucleotide sequence of two DNA classes that encode TFIID in *rabidopsis thaliana*. *a*, DNA and educed amino-acid sequence of At-1 DNA clones. Solid arrowheads indicate e positions of introns in the sequence f the At-1 genomic clones. Translation tart and stop codons are underlined. DNA and deduced amino-acid equence of At-2 cDNA clones. Trans- ition start and stop codons are under- ned.

ETHODS. A 680-base pair (bp) *NdeI*–*indIII* fragment of the clone GemIID8[17,23] containing the majority f the amino-acid coding region of the east TFIID gene was labelled by nick ranslation and used to screen a enomic library of *A. thaliana*. Hybridiz- tion was performed at 20% for- amide, 5×SSC, 50 mM Tris-HCl (pH .5), 1% SDS, 10× Denhardt's solution nd 50 μg ml^{-1} denatured herring perm DNA for 16 h at 37 °C. After ybridization, filters were washed in 2× SC, 1% SDS, twice for 5 min at room emperature and twice for 30 min at 7 °C. This screen yielded four positive lones which were all derived from the ame locus. Poly(A)$^+$ RNA of whole *A. haliana* grown under continuous white ght was used to construct an ligo(dT)-primed cDNA library in λZap Stratagene) using the cDNA Synthesis ystem Plus (Amersham). 750,000 laques of the primary library were creened under the same conditions s described above for the genomic lones, except that a fragment contain- ng the At-1 TFIID gene was used as a robe. In total, 20 positive clones were btained in this screen. After *in vivo* excision, nine of these clones, as well s subclones of the genomic fragments ybridizing with the yeast TFIID probe, were subjected to double-stranded sequencing using the Sequenase sequencing kit (USB). Sequence data were analysed with the DNASIS and ROSIS programs (Hitachi) on an IBM S/2 computer.

a

```
    1                                                               CG CAG ATC ACA AAT    14
   15  CTC ATC CCC AGA GAG AGA ACC CAG AGA GCG ATA TTG AAA ATC AAA ACT CTC TCC TTT ATA    74
   75  TAT AAT CCC AAT TTA CAA ATC TCT TTC CCT CTC TAA AAA TTT CTT ATC TTT GTA TAA AAA   134
  135  GCC TTC TCC TTT TTC AAA TCA TTC ACC TTC CTT TCG CCT TTT CGG GGG AAT TTC TTC CAA   194
                                                                                  ▾ (1)
  195  TCT GTT GGG TTT ACG AAA CCC TAG ATT TCG AAA TTC CGG ATT GCT AAC TAG CGA AAG AGA   254
  255  ATG GCT GAT CAA GGA ACG GAA GGG AGC CAG CCA GTT GAC CTT ACT AAA CAT CCT TCA GGG   314
    1   M   A   D   Q   G   T   E   G   S   Q   P   V   D   L   T   K   H   P   S   G      20
                             ▾ (2)
  315  ATT GTT CCT ACT CTT CAA AAT ATT GTC TCA ACA GTG AAC TTA GAC TGC AAG CTT GAT CTT   374
   21   I   V   P   T   L   Q   N   I   V   S   T   V   N   L   D   C   K   L   D   L      40
                                                                  ▾ (3)
  375  AAA GCC ATA GCT TTG CAA GCT AGG AAT GCT GAA TAT AAC CCC AAG CGT TTC GCT GCT GTA   434
   41   K   A   I   A   L   Q   A   R   N   A   E   Y   N   P   K   R   F   A   A   V      60
                                                                                  ▾ (4)
  435  ATC ATG AGG ATC AGA GAG CCA AAG ACC ACA GCG TTA ATT TTT GCT TCT GGG AAA ATG GTG   494
   61   I   M   R   I   R   E   P   K   T   T   A   L   I   F   A   S   G   K   M   V      80
                                                                          ▾ (5)
  495  TGT ACC GGA GCT AAA AGT GAA CAT CTT TCA AAG CTT GCT GCA AGA AAG TAT GCT CGG ATT   554
   81   C   T   G   A   K   S   E   H   L   S   K   L   A   A   R   K   Y   A   R   I     100
                                                      ▾ (6)
  555  GTT CAA AAG CTT GGC TTT CCT GCA AAG TTC AAG GAT TTT AAG ATA CAG AAC ATT GTA GGC   614
  101   V   Q   K   L   G   F   P   A   K   F   K   D   F   K   I   Q   N   I   V   G     120
  615  TCA TGT GAT GTC AAA TTC CCC ATT AGG CTT GAA GGT CTT GCA TAC TCT CAT AGT GCT TTC   674
  121   S   C   D   V   K   F   P   I   R   L   E   G   L   A   Y   S   H   S   A   F     140
              ▾ (7)
  675  TCA AGT TAC GAG CCT GAG CTA TTT CCA GGA TTG ATA TAT AGG ATG AAA CTT CCA AAG ATT   734
  141   S   S   Y   E   P   E   L   F   P   G   L   I   Y   R   M   K   L   P   K   I     160
                                                                          ▾ (8)
  735  GTA CTG CTT ATT TTT GTG TCA GGA AAG ATT GTT ATA ACT GGA GCC AAG ATG AGA GAA GAG   794
  161   V   L   L   I   F   V   S   G   K   I   V   I   T   G   A   K   M   R   E   E     180
  795  ACT TAT ACC GCA TTT GAG AAT ATC TAC CCA GTT CTT AGA GAA TTC AGG AAG GTC CAG CAA   854
  181   T   Y   T   A   F   E   N   I   Y   P   V   L   R   E   F   R   K   V   Q   Q     200
                  ▾ (9)
  855  TAA TAG TAT AGC CTA CCA TAT TGA AGG CAA AAA CCA TGT TCA CGT TTG GAT TGC CAT GAA   914
        *
  915  CTA GAG ATC TCG GGT GTT GGC AGG CGC TTG GTG GGG TTA TAT TCC TTG GAC AAG AAC TTG   974
  975  AAA TGA TTC AGA TTC AAA ACC AGA AAT TGT AAA TAG AGA ATG GAG ATG CCA ATA AAT CTC  1034
 1035  TTT TCT TTT TGT TCT AAA AAT GTC TTC CAA GTA ATC TTG TTT CAT GTT CTG TTT GTT TCA  1094
 1095  GTG AAC AAT ACC CAA TCA TTA AAG AGT TAC TTA GTT CTT TGC TAA CTG TTC TGT AAT AAG  1154
 1155  ACA CCA AGT CTG TTT GCC CTT TAA TAT ACT CTT TCA AGC                               1193
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b

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    1                   CA CAG ATC TAA AAA CCA GAA GCG AGA GAA ACA AAT TCA CTC TCC CCT    47
   48  TAT ATA AGC ACC GAT TTA TAA ATC TTT TTC CCT CTT CGA TTC TCA ATT CTT TGT ATA AAA   107
  108  GGC TTC TCC TTT TCT CAA TTC TTC GGC TTC CTT TCG CCC AAA CTC TTC CCT CGA ATC TTT   167
  168  CCT TCT CGT CTT AAA GCT ACG AAA CCC TAG ATT TCG GAT TTC TTC GCT ATC CAA AGA AGA   227
  228  ATG ACT GAT CAA GGA TTG GAA GGG AGT AAT CCA GTT GAT CTT AGC AAG CAT CCT TCA GGG   287
    1   M   T   D   Q   G   L   E   G   S   N   P   V   D   L   S   K   H   P   S   G      20
  288  ATT GTT CCT ACT CTT CAA AAC ATT GTC TCC ACG GTG AAC TTA GAC TGC AAG CTA GAT CTT   347
   21   I   V   P   T   L   Q   N   I   V   S   T   V   N   L   D   C   K   L   D   L      40
  348  AAA GCC ATA GCT TTG CAG GCT CGG AAT GCT GAA TAT AAT CCC AAG CGT TTT GCT GCG GTG   407
   41   K   A   I   A   L   Q   A   R   N   A   E   Y   N   P   K   R   F   A   A   V      60
  408  ATA ATG AGG ATC AGA GAA CCG AAG ACT ACA GCA TTA ATA TTC GCC TCA GGG AAA ATG GTC   467
   61   I   M   R   I   R   E   P   K   T   T   A   L   I   F   A   S   G   K   M   V      80
  468  TGT ACT GGA GCT AAG AGC GAG GAC TTT TCG AAG ATG GCT GCT AGA AAG TAT GCT AGG ATT   527
   81   C   T   G   A   K   S   E   D   F   S   K   M   A   A   R   K   Y   A   R   I     100
  528  GTG CAG AAA TTG GGA TTC CCT GCA AAA TTC AAG GAT TTC AAG ATT CAG AAT ATT GTA GGT   587
  101   V   Q   K   L   G   F   P   A   K   F   K   D   F   K   I   Q   N   I   V   G     120
  588  TCT TGT GAT GTC AAA TTC CCT ATA AGA CTT GAA GGT CTT GCT TAC TCT CAC GCT GCT TTC   647
  121   S   C   D   V   K   F   P   I   R   L   E   G   L   A   Y   S   H   A   A   F     140
  648  TCA AGT TAT GAG CCC GAG CTC TTC CCA GGG CTG ATT TAT AGG ATG AAA GTC CCA AAA ATC   707
  141   S   S   Y   E   P   E   L   F   P   G   L   I   Y   R   M   K   V   P   K   I     160
  708  GTC CTT CTA ATC TTT GTC TCT GGG AAG ATC GTA ATA ACA GGA GCC AAG ATG AGA GAT GAG   767
  161   V   L   L   I   F   V   S   G   K   I   V   I   T   G   A   K   M   R   D   E     180
  768  ACC TAC AAA GCC TTT GAG AAT ATA TAC CCC GTG CTC TCG GAA TTC AGA AAG ATA CAG CAA   827
  181   T   Y   K   A   F   E   N   I   Y   P   V   L   S   E   F   R   K   I   Q   Q     200
  828  TAG GTC ACG GAT TTG TTC CCT GCA AAA CTA GTT GTG CGG TCT TAG CCC CTT GGA GTT GCT   887
        *
  888  AAA GCT TGC TGA GAA TTT TGC CCT TGA ACA AAG GCT TTC ACA GTA GCT AGA CTC TCA CCT   947
  948  TGT GTT TTG GTT CAT AAT ATA ACA TTG TAT ATA CAC AAT GGA GAT TCT AAA GAC AAT TCT  1007
 1008  TCG GTT CTA TTT TTT TTC TTT TTC TTT CCA AGA TAT GTC TTA CAT GTA TGT TGC TGT AGT  1067
 1068  GTC CTC ACA TTT CCA CTT ATG TTA CAA GTA GAA CCT TAA CTC                           1109
```

screened a primary *A. thaliana* cDNA library under low stringency hybridization conditions using one of the genomic clones as a probe. We obtained 20 positive clones (out of 7.5×10^4) of which nine were analysed further by sequencing. Seven of these cDNA clones (*A. thaliana* type 1, At-1) correspond to the isolated *A. thaliana* genomic clones (Fig. 1*a*), whereas the remaining two cDNA clones (*A. thaliana* type 2, At-2) contain a very similar but clearly distinct sequence (Fig. 1*b*). Genomic Southern analysis using the At-1 and At-2 cDNA clones as probes confirms that there are at least two closely related genes present in the *Arabidopsis* genome (data not shown). Northern analysis using gene-specific probes reveals that the At-1 and At-2 messenger RNAs are 1.4 kilobases (kb) and 1.3 kb in size, respectively and that they are present in roughly equal amounts in whole light-grown *Arabidopsis* plants (data not shown). A sequence comparison between the At-1 cDNA and genomic clones reveals the presence of nine introns, the positions of which are indicated in Fig. 1*a*. The longest open reading frame in both types of cDNA clones encodes a protein of 200 amino acids, highly homologous to, but 40 amino acids smaller than, yeast TFIID. The calculated relative molecular mass (M_r) is 22,395 for *Arabidopsis* TFIID-1 and 22,367 for *Arabidopsis* TFIID-2.

To determine whether both cDNA clones encode a functional TFIID we tested TATA box-binding and basal transcription activities of *in vitro* expressed proteins. For At-1 as well as At-2 a protein of M_r 24,000 in size was obtained (Fig. 2*a*), matching the calculated size. Both proteins bind specifically to the adenovirus major late promoter in gel retardation assays (Fig. 2*b*). Both types of TFIID give three shifted complexes (termed 1 and 1′, 2, 3), suggesting that proteins in the reticulocyte lysate either modify or interact with the two proteins. Interestingly, complex 1 formed in the presence of the TFIID-1 is slower in mobility than complex 1′ formed in the presence of the TFIID-2, although both proteins have approximately the same molecular weight. This could indicate an interaction of TFIID-1 with proteins present in the reticulocyte lysate. Alternatively, the complexes of TFIID-1 and TFIID-2 bound to DNA could have different structures as a result of either a difference in the secondary structure of the two proteins or a difference in the way they bind to DNA.

To study further the function of both types of *Arabidopsi* TFIID, we expressed the two TFIID cDNAs in *Escherichia co* (Fig. 2*c*). Crude extracts of cells expressing these proteins wer then tested for TFIID activity in a reconstituted human *in vitr* transcription system containing RNA polymerase II and th basic transcription factors except TFIID. We used a templat that contains the adenovirus major late promoter fused to guanosine-free cassette[22]. Extracts of cells expressing TFIID- or TFIID-2 are both able to confer basal transcription in thi assay whereas extracts of cells containing the expression vecto alone have no activity (Fig. 2*d*). Taken together, these result clearly demonstrate that both cDNA clones encode functiona TFIID as both proteins exhibit a specific binding activity to th TATA box and can substitute for the human TFIID to activat basal transcription *in vitro*.

Figure 3 compares the deduced amino-acid sequences o *Arabidopsis* TFIID-1 and TFIID-2. Out of 200 residues, 18 (93.5%) are identical between the two proteins. The N-termina region (amino acids 1-18) is less conserved (75% identity) tha the rest (19-200) of the protein (95% identity). Figure 3 als compares the sequences of both *Arabidopsis* TFIID protein with that of the yeast TFIID. The N-terminal domain is completely divergent in sequence and smaller in size (18 compare with 60 residues) between *Arabidopsis* and yeast TFIID Moreover, the N-terminal domain of yeast TFIID is dispensabl for basal level transcription[23] suggesting that it is involved i contacting species-specific regulatory factors. The large C terminal domains, however, are highly conserved between th *Arabidopsis* and yeast TFIID proteins (85% amino acid sequenc identity). The high sequence conservation suggests that thi domain is involved in the highly conserved TFIID properties such as TATA box-binding and interaction with the genera transcription machinery. Indeed, these properties have bee mapped to the C-terminal residues 63-240 of yeast TFIID[23] This TFIID domain harbours several previously describe sequence motifs, all highly conserved between the *Arabidopsi* and yeast TFIID proteins: a domain rich in basic residues[17], a region of similarity to bacterial sigma factors[17] and a recentl discovered direct repeat[21]. Possible functions of these sequenc motifs, which are schematically shown in Fig. 4, are discussed in the accompanying manuscript[24]. Recently it was shown tha

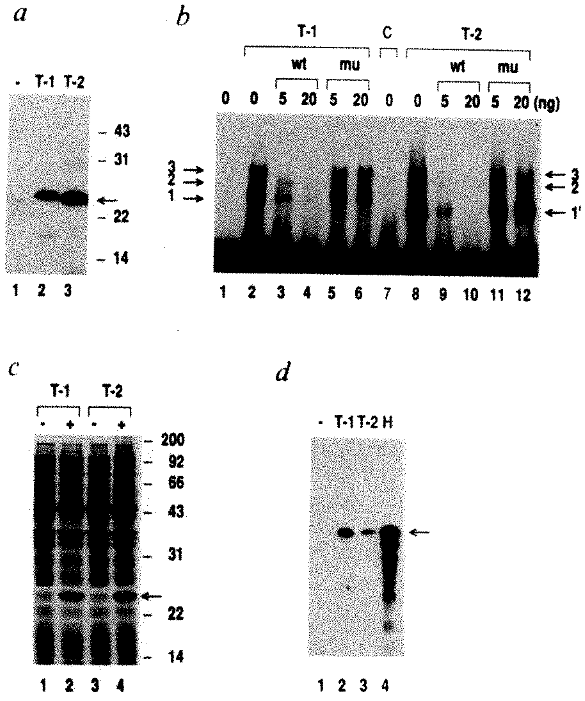

FIG. 2 Functional analysis of *Arabidopsis* TFIID-1 and TFIID-2. *a*, SDS-PAGE of $[^{35}S]$methionine-labelled TFIID-1 (T-1) and TFIID-2 (T-2) obtained by *i vitro* translation. Samples contained reticulocyte lysate programmed with no (−) RNA (lane 1), with At-1 RNA (lane 2), with At-2 RNA (lane 3). The arrow indicates the TFIID proteins. *b*, Site-specific DNA binding of TFIID-1 (T-1) and TFIID-2 (T-2). An adenovirus major late promoter fragment[17] was used for the gel shift assay[17]. The reactions included: no protein (lane 1) reticulocyte lysate programmed with At-1 RNA (lanes 2–6), with no (C) RNA (lane 7), with At-2 RNA (lanes 8–12). Binding was competed with 5 ng (lanes 3, 9), and 20 ng (lanes 4, 10) of wild-type (wt) TATA box sequence (TATAAAA) competitor DNA[17] or with 5 ng (lanes 5, 11), and 20 ng (lanes 6,12) of mutant (mu) TATA box sequence (TAGAGAA) competitor DNA[17]. *c*, SDS-PAGE of TFIID-1 (T-1) and TFIID-2 (T-2) expressed in *E. coli*. The lanes contain *E. coli* extracts carrying pET3a[26] with At-1 cDNA (lanes 1, 2), or At-2 cDNA (lanes 3, 4), either before (−) or after (+) induction with IPTG. The arrow indicates the position of TFIID. *d*, Activation of *in vitro* transcription by TFIID-1 (T-1) and TFIID-2 (T-2) in a reconstituted HeLa *in vitro* transcription system. Added were extracts of induced cells containing pET3A[26] without (−) insert (lane 1), with At-1 cDNA (lane 2) or At-2 cDNA (lane 3). In lane 4 partially purified human (H) TFIID[1] was added. The arrow indicates transcripts of 380 nucleotides.

METHODS. The At-1 and At-2 cDNA clones were transcribed *in vitro* using T3 RNA polymerase and T7 RNA polymerase (Stratagene), respectively. *In vitro* translation, SDS-PAGE analysis and gel retardation assays were performed as described[17]. Expression in *E. coli*, preparation of crude extracts and transcription assays were performed as described[27].

yeast TFIID does not form homodimers[23]. As most DNA-binding proteins bind DNA as dimers, it is tempting to speculate that intra- or intermolecular interaction of the direct repeats present in TFIID is necessary for DNA binding. We therefore directly compared the sequence of these TFIID repeats with sequence motifs known to mediate intermolecular interaction of DNA-binding proteins. Surprisingly, we found a weak similarity of both repeats to the helix-loop-helix domain (Fig. 4). This similarity is mostly confined to the residues that are thought to form the hydrophobic face of the helix 2 region in these proteins[25]. Our suggestion that these two regions in TFIID (residues 70-85 and 161-176) form α-helices can offer an explanation of how the direct repeats within the same TFIID molecule might interact—through the hydrophobic faces of these two putative helices. The sequence homology between TFIID and helix-loop-helix proteins also raises the exciting possibility that these proteins may interact through their amphipathic helix to form heterodimers. Such protein-protein interaction could be one mechanism by which the regulatory effect of helix-loop-helix proteins is transduced.

Our most important result is the finding of two distinct TFIID proteins. Previous reports have shown a heterogeneity among eukaryotic TATA elements: in yeast, different TATA elements are required for constitutive and inducible expression of the *his3* gene[9-11]. Alteration of the TATA element of several mammalian promoters results in dramatic restriction in inducibility, with only a subset of factors being able to activate the promoter[12-15]. Taken together, these results suggested the existence of functionally different TATA factors. To execute its proper function *in vivo*, TFIID has to contact three important components: the TATA-box, the general transcription machinery and regulatory factors. Distinct forms of TFIID are therefore likely to differ in their contact to at least one of these structures. We believe that *Arabidopsis* TFIID-1 and TFIID-2

```
A.t. TFIID-1   1                                             A   T   Q    T        18
A.t. TFIID-2   1                                            MTDQGLEGSNPVDLSKHP     18
S.c. TFIID     1  MADEERLKEFKEANKIVFDPNTRQVWENQNRDGTKPATTFQSEEDIKRAAPESEKDTSAT     60

A.t. TFIID-1  19                                                                   78
A.t. TFIID-2  19  SGIVPTLQNIVSTVNLDCKLDLKAIALQARNAEYNPKRFAAVIMRIREPKTTALIFASGK     78
S.c. TFIID    61             A  T G R    TV  H                                    120

A.t. TFIID-1  79           HL  L                                             S    138
A.t. TFIID-2  79  MVCTGAKSEDFSKMAARKYARIVQKLGFPAKFKDFKIQNIVGSCDVKFPIRLEGLAYSHA    138
S.c. TFIID   121    V       D  L S      I  I  A   T                       F  G    180

A.t. TFIID-1 139                    L                     E   T         R    V  * 200
A.t. TFIID-2 139  AFSSYEPELFPGLIYRMKVPKIVLLIFVSGKIVITGAKMRDETYKAFENIYPVLSEFRKIQQ* 200
S.c. TFIID   181  T                VK               L   Q E I Q   A          M*   240
```

FIG. 3 Sequence alignment of *A. thaliana* TFIID-1, TFIID-2 and *S. cerevisiae* TFIID. The *S. cerevisiae* (S.c.) TFIID sequence is taken from Horikoshi *et al.*[17]. The amino-acid sequence of the *A. thaliana* (A.t.) TFIID-2 is used as the reference. Only non-conserved amino acids between *S. cerevisiae* TFIID and *A. thaliana* TFIID-2 and between the two types of *A. thaliana* TFIID are shown. The last amino acid residue of each protein is followed by an asterisk.

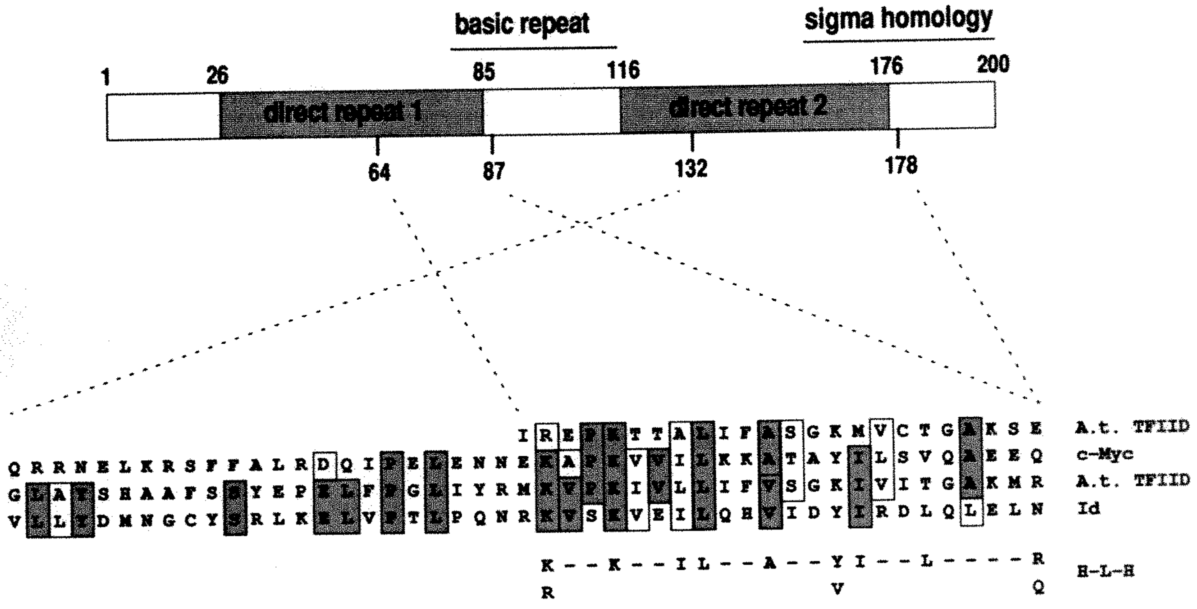

FIG. 4 Regions of similarity between TFIID and helix-loop-helix proteins. Upper panel: A schematic diagram of the structure of *Arabidopsis* TFIID-1 and TFIID-2. The direct repeat 1 encompasses residues 26-85 and the direct repeat 2 encompasses residues 116-176. Lower panel: row 1, amino acid sequence between residues 64-87 of *Arabidopsis* TFIID-1 and TFIID-2; row 2, amino-acid sequence of human c-Myc[28] (residues 357-403); row 3, amino-acid sequence of *Arabidopsis* TFIID-2 (residues 132-178). *Arabidopsis* TFIID-1 has the same sequence with the exception of a serine at position 138 and a leucine at position 157; row 4, amino-acid sequence of Id[29] (residues 85-131); row 5, consensus sequence of the corresponding region in helix-loop-helix (H-L-H) proteins[30]. Identical amino-acid residues in all four sequences are shaded. Conservative amino-acid exchanges are indicated by open boxes.

can be distinguished by their affinity to one or more of these components. The positions of amino-acid exchanges between TFIID-1 and TFIID-2 could provide some clue to where possible functional differences might be encoded. Further experiments are needed to determine exactly to what degree both factors differ in function and to pinpoint the residues that are important in these differences. ☐

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Spontaneous shuffling of domains between introns of phage T4

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THE three self-splicing introns in phage T4 (in the *td*, *sun*Y and *nrd*B genes) (Fig. 1*a*) each have the conserved group I catalytic RNA core structure (Fig. 1*b*), out of which is looped an open reading frame[1]. Although the core sequences are very similar (~60% identity), the open reading frames seem to be unrelated. Single crossover recombination events between homologous core sequences in the closely linked *td* and *nrd*B introns have led to 'exon shuffling'[2]. Here we describe spontaneous double crossovers between the unlinked *td* and *sun*Y introns that result in shuffling of an intron structure element, P7.1 (refs 3 and 4). The intron domain-switch variants were isolated as genetic suppressors of a splicing-defective P7.1 deletion in the *td* intron. This unprecedented example of suppression through inter-intron sequence substitution indicates that the introns are in a state of genetic flux and implies the functional interchangeability of the two analogous but no identical P7.1 elements. The implications of such recombinati events are discussed in the light of the evolution of the intro themselves as well as that of their host genomes.

To address questions of intron function and evolution v have isolated Td$^+$ pseudorevertants from Td$^-$ phage containi stable splicing-defective intron mutations. The ΔP7.1 deleti (Fig. 1*c*), which was introduced into a functional 265-nucleoti (nt) *td* mini-intron[5] (Fig. 1*b*) and shown to inhibit splicing (Schroeder, unpublished observations), was crossed into t phage, allowing selection for Td$^-$ plaques[6,7]. Subsequently, T plaques[7,8], which arose spontaneously from these T4*td*ΔP7 phage at a frequency of ~10^{-7}, were isolated and characterize by plaque hybridization (not shown) and sequence analysis (Fi 2). Remarkably, four independent Td$^+$ pseudorevertants ha acquired the P7.1 element from the *sun*Y intron (Figs 1*c* an 2). Although the P7.1 elements of *td* and *sun*Y differ (stems 4 versus 5 base pairs (bp) and loops of 4 versus 10 nt, respe

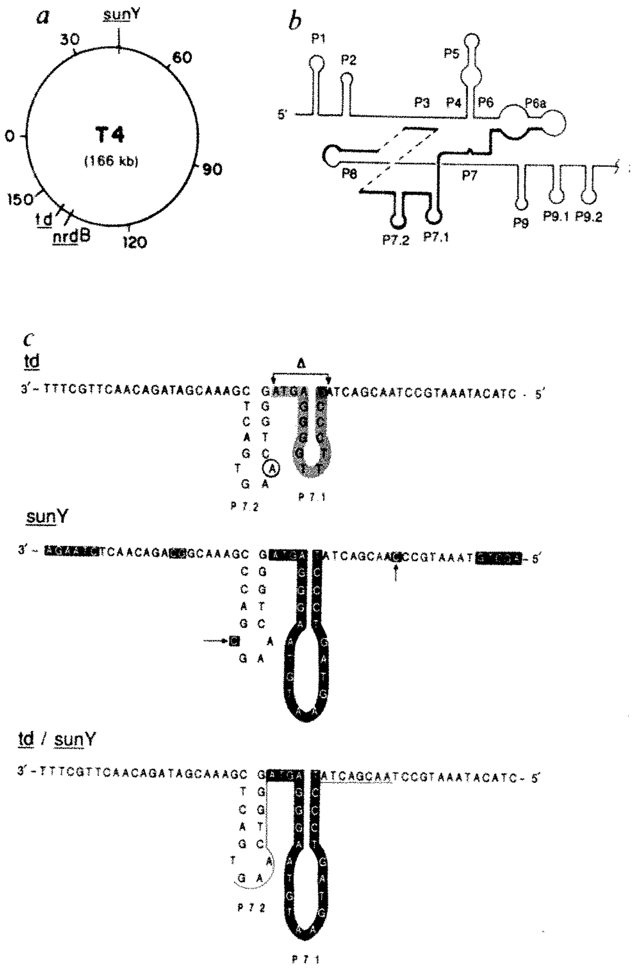
a
sunY
30
60
0
T4
(166 kb)
90
150
td
nrdB
120
b
P1
P2
P5
5'
P3
P4
P6
P6a
P8
P7
P9
P9.1
P9.2
P7.2
P7.1
c
td
Δ
3'- TTTCGTTCAACAGATAGCAAAGC GATGA TATCAGCAATCCGTAAATACATC - 5'
P7.2
P7.1
sunY
3'- AGAAATTCAACAGAATGCAAAGC GATGA TATCAGCAAGCCGTAAATGTCGA- 5'
td / sunY
3'-TTTCGTTCAACAGATAGCAAAGC GATGA TATCAGCAATCCGTAAATACATC- 5'
P7.2
P7.1

FIG. 1 *a*, Map of the circularly permuted T4 genome. The positions of the intron-containing *td*, *nrd*B and *sun*Y genes are shown on the 166-kilobase (kb) map, calibrated in kb. *b*, Secondary structure map of the *td* intron. The pairing elements (P1–P9.2) are shown[1,4], with the endonuclease-encoding ORF—naturally looped out of P6a—deleted[5], as it is in all *td*-containing phage and plasmids used in this work. The bold line indicates the sequences presented in *c*. *c*, Sequences of the P3–P7 region. The *td* sequence (top) shows the P7.1 deletion in T4*td*Δ7.1 shaded and bracketed by arrows (Δ). In the *sun*Y sequence (middle), nucleotides present in *sun*Y but not in T4*td*Δ7.1 are shown on a black background. Arrows point to the nucleotides that define the recombination boundary. The T4*td*–*sun*Y7.1 hybrid (bottom) has the nucleotides over which the double crossover event occurred marked by thin lines. These homologies are shown here as 8 nt on each side of P7.1, but could be drawn as 6 nt (5′) and 10 nt (3′) as there is a redundant AT sequence on both sides of P7.1. The circled A in the *td* sequence represents the 3′ residue in the autoradiographs shown in Fig. 2.

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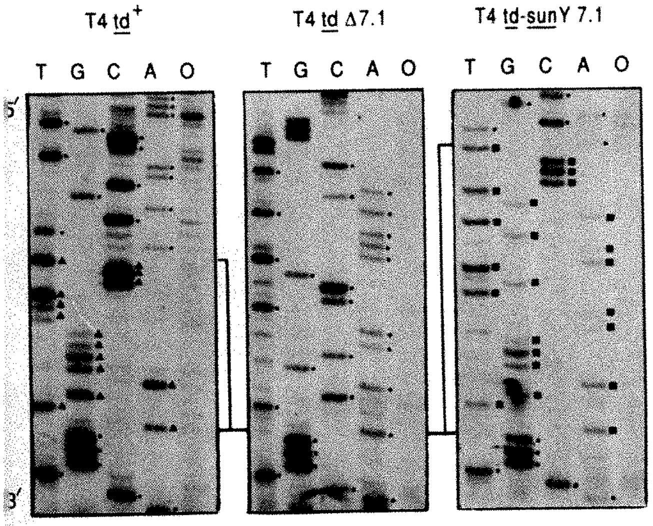
vely), they are flanked by homologous sequences (~8 nt on ach side; Fig. 1*c*, legend). Residues unique to *sun*Y immediately and 3′ to these stretches of homology define the outer limits f the double crossover events (Fig. 1*c*).

Surprisingly, the *sun*Y introns of the four hybrid variants, 4*td*-*sun*Y7.1, maintained their natural P7.1 element rather an having inherited the P7.1 deletion of *td* (data not shown). his result indicates that if the *td* intron acquired P7.1 of *sun*Y rom the same phage it must have occurred by an unusual onreciprocal recombination event between the two distant loci for example, intrachromosomal copy choice). Alternatively, if e translocation involved a straightforward double reciprocal rossover, it must have occurred between the introns of two ifferent phages (Fig. 3), implying that introns could serve as lements that align independent genomes to facilitate genetic ecombination.

Sequences between the P3 and P7 pairings (for example, P7.1 nd P7.2) are characteristic of subgroup IA introns[3,9]. All three T4 introns have P7.1 and P7.2 pairings[1], which seem to be important for intron function, as splicing is reduced when they are mutated or absent[10,11]. It was therefore of interest to evaluate the function of the hybrid intron in a Td$^+$ T4*td*-*sun*Y7.1 variant. *In vivo* activity was monitored by determining pre-messenger



IG. 2 Sequence of the P7.1 variants. RNA extracted from T4-infected scherichia coli B nine minutes after infection at 37 °C was sequenced by he dideoxy-chain-termination method with an intron-specific primer[17]. Lanes are labelled with the complement of the ddNTP used. ▲, 15 nt encompassing he P7.1 element that are deleted from T4*td*Δ7.1; ●, nucleotides shared y all the *td* variants; ■, *sun*Y sequences acquired by T4*td*Δ7.1 (23 nt) to ield T4*td*–*sun*Y7.1. Residues deleted from T4*td*$^+$ and those inserted in T4*td*–*sun*Y7.1 are bracketed relative to T4*td*Δ7.1. The sequences are resented in Fig. 1*c*, with the 3′ residue (bottom of the autoradiograph) ircled in Fig. 1*c*.

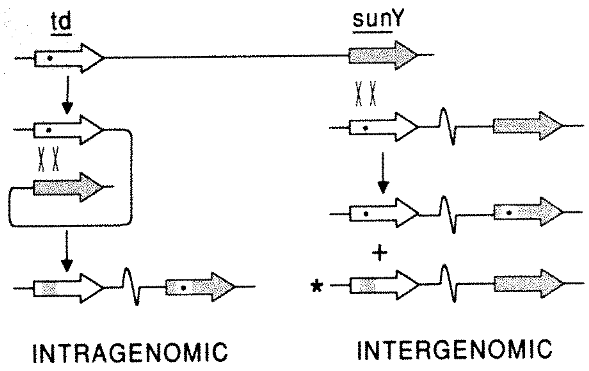


FIG. 3 Interchromosomal versus intrachromosomal reciprocal recombination. Large arrows represent homologous introns as indicated. ●, P7.1 deletion. ××, double reciprocal crossovers. The interchromosomal pathway on the right could account for the observed recombination product (*), with the translocated *sun*Y sequences having been preserved in the *sun*Y intron of the same genome.

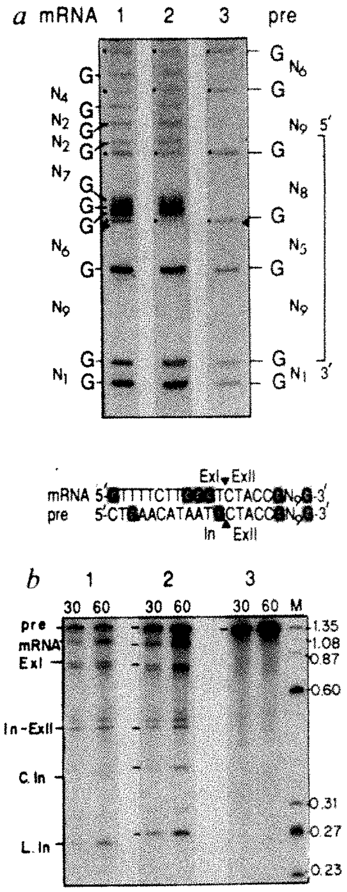


FIG. 4 Functional analysis of the *td* variants. *a*, Splicing *in vivo*. RNA extracted from cells infected with T4*td*$^+$ (lane 1), T4*td*–*sun*Y7.1 (lane 2) and T4*td*Δ7.1 (lane 3) 9 min post-infection was analysed by primer-extension[17]. A 19-nucleotide primer, which anneals to exon II 86 nt from the 3′ splice site, was extended in the presence of ddCTP. By causing chain termination at complementary G residues in the RNA template, this analogue gives a characteristic pattern of stops for mRNA versus pre-mRNA. The characteristic G residues are indicated for mRNA and pre-mRNA (pre) alongside the autoradiograph, with the number of intervening residues marked (N). Sequences (G residues shaded) around the exon I–exon II junction of mRNA and the intron–exon II junction of the pre-mRNA are shown below for the region bracketed on the right of the autoradiograph. The pattern of G residues is identical in exon II (bottom of autoradiograph) and diverges after the junction (►). By scanning the autoradiograph, pre-mRNA-specific bands (●) were compared with mRNA-specific bands. Reactions were run in parallel in the presence of ddATP, ddGTP and ddTTP, to calibrate the gels and to confirm the fidelity of splicing (not shown). *b*, Splicing *in vitro*. The following plasmids—linearized in exon II with *Eco*RV—were used as templates for *in vitro* transcription with T7 polymerase and [α-^{32}P]GTP (Stratagene) under conditions recommended by the supplier, for 30 or 60 min as indicated: pTZ*td*$^+$ (panel 1), pTZ*td*–*sun*Y7.1 (panel 2) and pTZ*td*Δ7.1 (panel 3). Plasmid pTZ*td*–*sun*Y7.1 was generated by an *in vivo* cross (15 min at 37 °C in RRIΔ*thy*A::KanR cells) between Td$^-$ plasmid pTZ*td*ΔP6.13 (ref. 5) and Td$^+$ phage T4*td*–*sun*Y7.1, followed by selection for progeny plasmids with a Td$^+$ (Thy$^+$) phenotype[10]. Labelling of the autoradiograph is as follows, with sizes of the wild-type *td* RNAs in parentheses: pre, presursor (1,237 nt); mRNA, ligated exons (973 nt); ExI, free exon I, (770 nt); In–ExII, intron-exon II (468 nt); C.In, circular intron (264 nt) and L.In, linear intron (266 nt). Because of efficient use of a cryptic splice site in exon I (ref. 18), mRNA, ExI, In–ExII and L.In appear as doublets. Lane M contains ϕX174/*Hae*III DNA size markers. The intron in all constructs has the 751-nt ORF deletion[5], leaving an intron of 265 nt for *td*$^+$, 273 nt for *td*–*sun*Y and 250 nt for *td*Δ7.1 constructs (note appropriate migration differences between panels 1 and 2).

RNA/messenger RNA ratios of *td* transcripts from phage-infected cells, using a primer-extension assay. Thus, T4*td*Δ7.1 was shown to be splicing-defective, whereas T4*td-sun*Y7.1 was shown to undergo high levels of accurate splicing (Fig. 4*a*). To assess self-splicing proficiency, the hybrid intron was crossed into an *in vitro* transcription vector. Plasmids containing the wild-type *td* or *td-sun*Y7.1 introns conferred a Thy$^+$ (Td$^+$) phenotype on Thy$^-$ cells[10], whereas those with the *td*Δ7.1 intron did not. Accordingly, *td*Δ7.1 precursors did not function *in vitro*, whereas *td*$^+$ and *td-sun*Y7.1 pre-mRNA spliced efficiently (Fig. 4*b*). Although subtle differences in splicing activity have not been ruled out, these results suggest that the different P7.1 elements of the *td* and *sun*Y introns are functionally interchangeable.

The type of ectopic crossover described here would influence only the intron core structure and therefore splicing. However, parallel events involving sequences flanking an open reading frame (ORF) could have a profound influence on the composition and ultimate fate of the intron. As both the *td* and *sun*Y ORFs encode endonucleases that promote intron mobility[7,12,13], recombinational 'ORF-shuffling' could result in either limiting or extending intron spread.

Double crossovers between separated elements extend the range of viable recombinational possibilities because they leave undisturbed the regions between the homologous elements. By contrast, single crossovers lead to major genetic rearrangements between repeated elements[14] and are therefore confined to introns that span non-essential regions. Such events are exemplified by hybrids of the closely linked *td* and *nrd*B introns, which have undergone deletion of the dispensable sequences between them[2]. Because double crossovers introduce discrete genetic replacements in the homologous elements they can occur between distant loci without threatening viability, as demonstrated here for the unlinked *td* and *sun*Y introns. Because as few as five homologous nucleotides are required at an inter-intron crossover locus (M.B. and M.B., unpublished observations), similar double recombination events may also facilitate exchanges involving exons (with one crossover within homologous introns, the other in flanking exons). Although the latter events remain to be demonstrated, the ability of unlinked introns to facilitate recombination over wide genetic distance is clear. The possibility that such crossovers might occur interchromosomally adds further interest to the hypothesis that introns act as 'recombinogens' that increase the opportunities for genetic exchange and thereby speed the evolution of genomes[15]. Boosting the evolutionary fitness of the phages may indeed provide the rationale for the maintenance of introns, which are without an apparent phenotype[16], in the reproductively efficient phages. Nevertheless, inter-intron sequence translocation must influence evolution of the introns themselves. ☐

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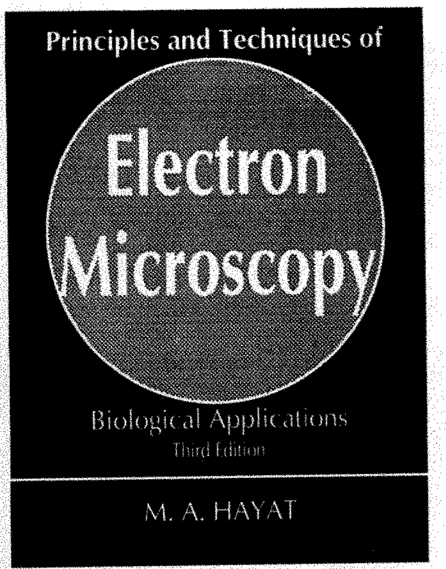

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The professor is responsible for teaching and research in the field of isotope geology. He must develop new research directions as well as teaching programs, and supervise Ph.D. students. He should promote and encourage collaboration with other research groups, both within and outside the university, in the field of long life radio-active and radiogene isotopes.

Requirements
Potential candidates must have outstanding scientific qualities at the international level in the field of isotope geology/geochemistry. Their background may be in chemistry, physics or geology, but they should have considerable recent experience in isotope geology. They should possess proven management and teaching qualities and the ability to stimulate and coordinate colleagues and students. Knowledge of the Dutch language is not initially required.

Salary
The salary will be according to the Dutch Civil Servants Code, with a maximum of Dfl. 10.544,- a month depending on age and experience (excluding 8% holiday allowance).

Information
Further information may be obtained from prof.dr. J.L.R. Touret, chairman nomination committee, telephone 020 - 548 2442 (private: 023 - 314549).
Applications with full curriculum vitae and the names of at least three references should be submitted before September 15th, 1990, to: Vrije Universiteit, secretaris/beheerder Faculteit der Aardwetenschappen, De Boelelaan 1085, 1081 HV Amsterdam, The Netherlands.

The objective of the Vrije Universiteit is to increase the percentage of females employed in the organization.
Members of staff are expected to respect the christian principles of the Vrije Universiteit. The campus of the Vrije Universiteit is on De Boelelaan in Amsterdam Buitenveldert.

(W7586)A

University of Otago

New Zealand

ASSISTANT LECTURER/LECTURERS IN CHEMISTRY

Applications are invited from women and men for two positions in the Department of Chemistry. The successful candidates will be expected to contribute to the undergraduate teaching programme in the Department and to be actively engaged in research.

Applicants should hold a higher degree in Chemistry with a sound background in physical or inorganic chemistry. It is anticipated that one appointee will have research experience in solution chemistry (colloids/electrolyte/acid-base) so that they can interact with staff in the biological sciences.

The appointment will be for three years in the first instance and salary will be within the Assistant Lecturers scale $NZ30,000 - $NZ34,500 or on the Lecturers scale at $NZ36,000 - $NZ43,700 per annum depending on qualifications and experience.

Enquiries may be made to Professor B.H. Robinson, Department of Chemistry, FAX NZ (024) 797-906, by telephoning NZ (024) 797-907 or E-Mail CHEMMAIL%Otago.AC.NZ@relay.cs.net.

Applications quoting reference A90/39 close with the Registrar, P.O. Box 56, Dunedin, New Zealand on 21 September 1990.

Equal opportunity in employment is University policy.

MAF

MINISTRY OF AGRICULTURE AND FISHERIES
TE MANATU AHUWHENUA AHUMOANA

PLANT EXTRACTS RESEARCH UNIT RESEARCH COORDINATOR

Applications are invited from women and men for the position of Research Coordinator in the Plant Extracts Research Unit situated in the Department of Chemistry, University of Otago.

Applicants should hold a PhD degree with a proven research record in natural product chemistry. Experience in modern analytical techniques is essential and a knowledge of the fragrance, flavour or drug industry could be an advantage.

The appointment will be for two years in the first instance and salary will be within Level 6 of the MAFTech scale depending on qualifications and experience.

Enquiries may be made to Professor B.H. Robinson, Department of Chemistry, FAX NZ (024) 797-906, by telephoning NZ (024) 797-907 or E-Mail CHEMMAIL%Otago.AC.NZ @relay.cs.net.

Applications on Form PS17A (available from any Government Department or the Department of Chemistry, University of Otago) quoting Vacancy No. MTS 90/13 together with curriculum vitae and the names of two referees should be sent to - Registrar, University of Otago, P.O. Box 56, Dunedin.

(W7596)A

Applications close on 24 August 1990.

MAF Technology has a policy of Equal Employment Opportunities.

IRELAND

AGRICULTURAL INSPECTOR (TOXICOLOGIST)

in the Department of Agriculture and Food

Essential:

In addition to holding a primary degree applicants must

— posses a post-graduate qualification in occupational medicine and/or toxicology;

— have had five years' post-graduate experience in hazard assessment of toxic chemicals, preferably pesticides, including experience of histopathological laboratory equipment and techniques.

Salary will be in the range:
IR£20,536—IR£27,075 Entry up to the maximum possible.

Closing date: 30th August, 1990.

Application forms and full particulars are available from the Secretary, Civil Service Commission, 1 Lower Grand Canal Street, Dublin 2.

The Civil Service Commissioners are committed to a policy of equal opportunity. (4250)A

UNIVERSITY OF OXFORD

PROFESSORSHIP OF EXPERIMENTAL PARTICLE PHYSICS

The electors intend to proceed to an election to the Professorship of Experimental Particle Physics with effect from 1st October 1991 or such later date as may be arranged. The Professor will lead one of the major experimental groups in this field in the UK, and may expect to serve (for periods of five years) as Head of the sub-department of Particle & Nuclear Physics, one of the six which together form the Department of Physics.

Applications (ten copies, or one from overseas candidates), naming three referees but without testimonials, should be received not later than 17th September 1990 by the Registrar, University Offices, Wellington Square, Oxford OX1 2JD, from whom further particulars may be obtained. (4228)A

The University is an Equal Opportunity Employer

UNIVERSITIES OF LEICESTER AND SHEFFIELD

NERC POST–DOCTORAL RESEARCH ASSOCIATES

PHOTOSYNTHETIC ACCLIMATION TO SHADE

Applications are invited for two post-doctoral research associates to take part in an new initiative between the Universities of Leicester and Sheffield sponsored by the Natural Environment Research Council. The project will consider the response of higher plant species to shade, seeking to elucidate the role of the phytochrome gene complement in the various morphological bio-chemical and physiological changes that constitute ecologically divergent shade strategies. The programme seeks to exploit the complementary skills, expertise and facilities in photomorphogenesis at Leicester and in photosynthesis at Sheffield and the research associates will be expected to work closely together. The project will initially be based at Leicester with subsequent transfer of parts of the research to Sheffield.

Molecular biology of phytochrome: For this post, experience of recombinant DNA techniques and plant transformation methodology is sought; an intimate knowledge of phytochrome is not essential as ample opportunity will be provided to acquire this expertise through working in a group of ca 10 research personnel in the Plant Photobiology Group in the Department of Botany at Leicester.

Photosynthesis: for this post, an individual with experience of the biochemistry or molecular biology of photosynthesis is sought. The successful applicant will be primarily based at Sheffield and will become a member of the Robert Hill Institute working in newly refurbished, well-equipped laboratories in the Department of Molecular Biology and Biotechnology.

Both positions are funded for three years with a starting salary at point 4 on the RAIA scale. For further details contact Professor Harry Smith, Department of Botany, University of Leicester (0533 523380) for the first post and Dr Peter Horton, Department of Molecular Biology and Biotechnology, University of Sheffield (0742 768555 ext 4189) for the second.

Applications to: **Professor Harry Smith, Department of Botany, University of Leicester, Leicester LE1 7RH** *with a full CV and the names of two referees; include fax number if available.*

Closing date for applications: 17th August, 1990. (4227)A

Advancing the understanding of pet ownership and nutrition.

Two unique career opportunities in the UK

The Waltham Centre for Pet Nutrition is one of the leading international authorities in its field. Located in England's rural East Midlands, it is in the forefront of studies into the nutrition and palatability of petfoods, and the behaviour and care of pet animals. The scope of its activities continues to expand, and high-calibre scientists with an international outlook are now sought to lead programmes of study in the following areas:

Pet ownership to £42,000 + benefits

You will initiate a wide-ranging programme of work in the key area of relationships between humans and pet animals, making an important scientific contribution to the case for increasing pet ownership worldwide. This innovative, project-oriented, non-routine position will involve international travel to external research institutes and other interested organisations; the business objective will be to identify areas of pet ownership offering maximum opportunity for Mars Petcare companies worldwide.

You will need a higher degree in a biological discipline plus some post-doctoral behavioural research experience. You should also be an effective, influential communicator who can quickly assess the potential value of creative lines of enquiry.

Avian nutrition to £29,000 + benefits

You will have wide scope to develop the Centre's nutritional expertise in respect of both caged and wild birds – a role which will involve initiating and controlling a programme of research studies, and providing information and support to Mars Petcare companies, academic institutions and other interested bodies.

You will need at least a good first degree in nutritional science or a related subject, preferably backed by some relevant post-qualification work in this area. A creative approach and good communication skills are essential.

Candidates who combine academic excellence with the personal drive and potential to make real career progress can anticipate good prospects of advancement: career development could involve a variety of business functions within Mars Petcare companies worldwide.

Salaries for both posts are backed by a valuable range of non-contributory benefits including a comprehensive international relocation package if required.

For more information and an application form, please telephone (UK) 0476 85796, ext 104, between 09.00 and 17.00 GMT, Monday to Friday. Overseas candidates can fax requests for details, specifying which post is of interest, to (UK) 0476 85795 (available 24 hours). Please do **not** send a cv at this stage. We welcome applications equally from women and men.

WALTHAM®
WORLD AUTHORITY ON
PET CARE & NUTRITION

(4281)A

Cancer Research Campaign

CRC GRAY LABORATORY

One further PhD studentship is available in the Institute which is funded entirely by the Cancer Research Campaign. Nine research groups are working on diverse aspects of radiation science and vascular biology, with the common aim of developing and improving cancer therapy. The CRC Gray Laboratory is set in the grounds of a hospital in the green belt on the outskirts of London and has close links with clinical work. The exceptionally good laboratory space and equipment and the wide spectrum of scientific expertise make this a unique and creative research environment. Academic links exist with both London and Brunel Universities. The studentship is in one of the following areas of research:-

BIOPHYSICS : Dr B.D. Michael

DNA damage in relation to cell kill; the role of free radical processes in DNA damage; microscopic interactions of various radiation types with cellular targets. Ref: GL2N/8

TUMOUR AND CELL RADIOBIOLOGY : Dr N.J. McNally

Interaction between damage caused by radiation and restriction enzymes, effect on DNA damage and its repair, and cell survival. New methods of using flow cytometry and monoclonal antibodies to quantify proliferating cells in tissues before and during radiotherapy. Ref: GL2N/1

CHEMISTRY AND BIOANALYSIS : Prof. P. Wardman

Chemical and biochemical factors controlling the activity of drugs which redox cycle, including bioreductive drugs for tumour therapy. Rates of drug reduction or oxidation in chemical and biochemical models, characterisation of free-radical intermediates and measurements of biochemical changes *in vitro*. Free-radical kinetics, pulse radiolysis, enzyme catalysis. Ref: GL2N/6

RADIOBIOLOGY APPLIED TO THERAPY : Dr M.C. Joiner

Influence of cellular proliferation on the response of tumours and normal tissues to radiation treatment. Evaluation of radiation treatments using multiple low dose-rate exposures. Direct cell killing, transformation and mutagenesis in cells exposed to low doses of ionizing radiation. Characterisation of cell subpopulation from tumours, using automated microscopy. Ref: GL2N/2

NEW TECHNOLOGIES IN PHYSICAL CHEMISTRY/BIOCHEMISTRY : Dr B. Vojnovic

Radical mechanisms underlying the action of redox compounds which kill tumour cells either through radiosensitisation or hypoxia - selective activation. Time-resolved studies using pulse radiolysis techniques. Ref: GL2N/9

The tax-free stipends for these studentships will start at £6,850.

Applicants should have at least an upper second class degree in a relevant subject. Further information about particular projects and a copy of our Annual Report can be obtained from the project supervisor listed above at: CRC Gray Laboratory, PO Box 100, Mount Vernon Hospital, Northwood, Middlesex HA6 2JR, (09274 28611). (4277)A

Fighting cancer on all fronts.

UNIVERSITY OF OXFORD

NUFFIELD DEPT OF CLINICAL MEDICINE

Institute of Molecular Medicine

POST DOCTORAL SCIENTIST – RS1A

Applications are invited for a post doctoral research fellow to join a recently formed group studying hypoxic regulation of the Erythropoietin gene. The group is based within the Institute of Molecular Medicine at the John Radcliffe Hospital and has close links with the MRC molecular haematology unit. We are using RNAse protection assays, competitive PCR, cell culture, transient transfection and transgenic experimentation in addition to classical physiological methods such as isolated kidney perfusion to address both the physiology and biochemistry of hypoxic sensing.

The post is funded for three years by the Wellcome Trust and includes an enhancement premium for this post, of three incremental points. A background in molecular biology would be desirable. *Salary scale: RS1A £10,458-£14,169. The University of Oxford is an Equal Opportunity Employer.*

Please apply in writing for an application form and job description quoting ref: GTW/RS1A/Ratcliffe.

Assistant Administrator, Nuffield Dept of Clinical Medicine, Room 5801, John Radcliffe Hospital, Headington, Oxford OX3 9DU.

Prospective candidates may telephone Dr Peter Ratcliffe 0865 752382 (direct line) to discuss the project.

Closing date for applications: 10th August, 1990. (4276)A

MICROBIOLOGY LABORATORY MANAGER

NORTH LONDON BLOOD TRANSFUSION CENTRE

THE POST:

Organising and planning the workload of a lively progressive laboratory

Managing and training staff

Participating in research and development

THE PERSON:

Educated to further degree level or having equivalent experience

Possessing good communication and inter-personal skills

Experienced in Blood Transfusion and/or Microbiology

Energetic and forward thinking

If you have the above qualities, drive and commitment we'd like to hear from you to help us continue to develop our staff and maintain our high standard of professionalism in the continually evolving environment of transfusion microbiology.

Salary 18-20K.

Apply to Personnel Department, NLBTC, Colindale Avenue, London NW9 5BG or telephone 081-905 9642 for a 24 hour answering service.

Please quote Ref no: 156PTA/90.

Closing date: 24th August, 1990. (4284)A

ST. LUKE'S CANCER RESEARCH FUND

FOUR POSTDOCTORAL SCIENTISTS

Four positions, each of three years duration, are available immediately for postdoctoral scientists to participate in studies of the medicinal chemistry and molecular pharmacology of DNA binding anticancer agents. Two of these positions are for synthetic chemists with skills in peptide, oligonucleotide or heterocyclic chemistry and two are for biochemists or pharmacologists with skills relevant to the study of mammalian transcription or drug binding to nucleic acids.

The object of the synthetic programme is to design and synthesise sequence selective ligands that have the potential to be specific inhibitors of gene expression. The successful chemistry candidates will work in the Chemistry Department at University College Dublin and at least one will have experience of, or interest in, computer-aided drug design methods.

The project has two biochemical aspects; the first concerns the effects of ligand binding to DNA on transcription in mammalian cells in culture and in cell-free systems, the second concerns studies of ligand binding to DNA and chromatin both in vitro and in vivo. The successful biochemical candidates will work in the Pharmacology Department at University College Dublin, one will have experience of the molecular biological techniques required to study mammalian transcription and the other will be experienced in the biochemical methods used to study DNA-ligand interactions and chromatin structure.

All four positions are tenable on annually-renewable contracts for a period of three years and would suit postdoctural scientists in the early stages of their careers. The salary range is IR£12,000-16,000 depending on age and experience. Applications should be addressed to Professor Laurence P.G. Wakelin, St. Luke's Hospital, Highfield Road, Rathgar, Dublin 6, Ireland (fax: 353 1 974 886), and include a curriculum vitae, publication list and names and addresses of three referees. Further information can be obtained by calling Professor Wakelin on 353 1 975 418. (4240)A

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NOVEL BIOSENSOR SYSTEMS

Protein Biochemistry/Laboratory Electronics

UK: To c. £19,000+car **France: To c. FF225,000+car**

Our client, a major force in the biotechnology and health care market, has established an exciting specialist venture taking new technology into the market place. We seek two well-qualified scientists to spearhead the development of this new venture in the UK, and one to do so in France.

The posts encompass a broad range of responsibilities: establishment of key customer relationships; technical user support; running seminars and workshops; and research feed-back from the market-place to the corporate R & D group. One of the UK posts will focus on the instrumentation itself, and the other on clients' applications.

Ideal applicants will be of high calibre and possess solid experience in protein biochemistry (for the applications specialist and the French post) *or* laboratory electronics (for the instrumentation specialist). Additional attributes should include:

- Familiarity with PCs and MS-DOS.
- Strong inter-personal skills and a "team player" attitude.
- Willingness to travel extensively in the UK/France and occasionally elsewhere, notably to Germany.
- Living — or willing to live — in the Northern Home Counties/Cambridge area for the UK positions and Paris for the French position (relocation package available).

Native French and fluent English are essential for the post based in France. For the UK posts, fluency in French and/or German would be advantageous. For all three posts industry experience (especially in sales) would also be desirable.

The posts all command an excellent remuneration package, dependent on qualifications and experience, together with a quality car.

For further information, please contact Pippa Anderson:

Talentmark
Leaders in Bioscience Recruitment

Talentmark Recruitment Consultants
King House, 5-11 Westbourne Grove
London W2 4UA. Phone: 071-229 4214.

(4275)A

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Cell Biologist/ Biochemist

Virology

The Research Centre at Welwyn Garden City maintains programmes of original, high quality Research and Development, contributing to the continued success of F. Hoffmann-La Roche as a major force in the worldwide pharmaceutical industry.

A new position has been created within our Chemotherapy Biology Department which is involved in the search for novel antiviral therapies. This position provides the opportunity to investigate the structure and function of viral proteins which are believed to be involved in cell transformation. The research to be undertaken will involve a close collaboration between Roche Research and the Ludwig Institute which is based at St Mary's Hospital in London.

The ideal candidate will be either a recently qualified PhD or an experienced graduate with expertise in the cell biology and/or biochemistry of cell transformation.

In return for your commitment and scientific contributions you will receive the benefits associated with a progressive organisation of international repute, including sound career and salary progression.

To apply, please write quoting current salary details to **Maxine Lambert, Roche Products Limited, PO Box 8, Welwyn Garden City, Herts AL7 3AY.** Alternatively telephone **(0707) 328128** for an application form.

Roche

Queen's Award for Export Achievement for [illegible]

(4267)A

Technology Transfer

BUSINESS MANAGER

Cancer Research Campaign Technology Ltd, a company wholly owned by the Cancer Research Campaign has a vacancy for a Business Manager. The individual appointed will assist in the exploitation and commercialisation of scientific and clinical developments arising from CRC-sponsored research.

Applicants should have a basic training in the medical physics or biological sciences (to at least first degree level) and several years experience in post-graduate research or industrial, legal or business experience relevant to technology transfer.

The appointment will be within the salary scale £16,000–£18,000 inclusive of London Weighting. There is a non-contributory pension scheme.

Applications, including a curriculum vitae and the names and address of two referees should be sent by 16 August 1990 to Sophie Smith, Personnel Assistant, Cancer Research Campaign, 2 Carlton House Terrace, London SW1Y 5AR.

Cancer Research Campaign

fighting cancer on all fronts

(4282)A

Royal Postgraduate Medical School

(University of London)

(Hammersmith Hospital)
DEPARTMENT OF VIROLOGY

Lecturer or Senior Lecturer

Applications are invited for a permanent (tenure-track) position in the Department of Virology. Appointment will be made either at Lecturer or Senior Lecturer level depending upon the qualifications and expertise of the applicant. Medically qualified persons may be appointed to an Honorary Consultant post in the Hammersmith Hospital. The ideal candidate will have a strong interest in basic research, some experience in molecular and cellular biology, and the desire to establish an independent scientific research programme in an area of medical-related virology. There is a teaching commitment in the Department and some experience in lecturing and training PhD students will be an advantage. The current interest in the Department centres around herpes — and oncogenic viruses, but applicants from other areas of virology are also welcomed. The Department is modern and well-equipped, and expansion is planned within 6-9 months to include a central service in molecular biology and tissue culture. Appointment may be made subject to a review or probationary period and salary will be appropriate to the qualifications and experience of the appointee.

Informal enquiries to Professor Beverly Griffin, Department of Virology, telephone 081-740 3248.

Letters of application, together with a full c.v. (7 copies) plus the name and addresses of 3 referees should be sent to the **Personnel Officer, Royal Postgraduate Medical School, Ducane Road, London W12 0NN (tel: 081 740 3204)** from whom further particulars are available. Ref: AV02

Closing date: 31 August 1990.

Laboratory Manager/MLSO 3/4

The Department of Virology at the RPMS has grown over the last few years to the point where it becomes desirable to establish a new position of **Laboratory Manager**. It is hoped to appoint at a senior level someone with a strong interest in laboratory science and management skills. There is considerable flexibility in the post, but preference will be given to a person with an interest and experience in microbiology, seeking a career that involves decision taking and responsibility. The department has an active interest in both clinical diagnosis and research into viruses associated with human disease, especially oncology. For details, contact the **Personnel Office, Royal Postgraduate Medical School, 150 Ducane Road, London W12 0NN or telephone 081-740 3204.** Salary scale, up to £17304 (+ £1580 London Allowance) MLSO 3 or exceptionally MLSO 4, depending upon qualifications and experience. The holder of this post will be directly responsible to the Head of the Department.

Closing date: 9th August, 1990.

(4237)A

MRC Clinical Research Centre
Watford Road
Harrow HA1 3UJ
Tel: 081.864 3232

POSTDOCTORAL SCIENTIST

Required to join a research group with an established programme investigating the pathogenesis of osteoporosis. Attention will be paid to fundamental mechanisms governing the maturation and biochemical functioning of osteoblasts and other cells regulating bone remodelling. Experimental techniques used will include quantitative histormorphometry and *in situ* cell biochemistry, integrated with a clinical programme of investigation. Patients with various forms of osteoporosis and other bone disorders are being studied using modern clinical techniques including dual x-ray absorptiometry and kinetic studies. Applicants should have broad interest in the programme but scientific expertise in all its aspects is not essential as appropriate training is available. Applicants should be with 3(4) years of their higher degree. Informal enquiries may be made by telephoning Dr Jonathan Reeve (081.869.3208/3197).

Salary will be on an appropriate point on the scales for non-clinical university academic staff. Application forms may be obtained from the Personnel Dept 081.869.3431 quoting ref 111/1/4221. Closing date for completed applications is 17th August, 1990. (4232)A

The Medical Research Council is an Equal Opportunity Employer.

INSTITUTE OF NEUROLOGY
QUEEN SQUARE

UNIVERSITY OF LONDON
MIRIAM MARKS DEPARTMENT OF NEUROCHEMISTRY

Applications are invited for a lectureship (non-clinical) in th
above department from 1st October, 1990. Applicants should hav
post-doctoral experience in Biochemistry or any closely related discipline. Preference will be given to candidates with a proven trac
record of substantial publication and obtaining grant income.

The Institute, which received a '5' rating in the recent UFC Research Selectivity Exercise, is part of the Bloomsbury campus of th
University of London.

Salary according to age and experience on either Lecturer A or
scale: £10,458-£15,372 or £16,014-£20,469 (pay award pending)
plus £1,767 London Allowance.

**Candidates should send a CV and the names of 3 referees to: Mis
E Bertram, Assistant Secretary (Personnel), Institute of Neurology, The National Hospital, Queen Square, London WC1
3BG, by 17th August, 1990. Informal enquiries and further details may be obtained from Professor J B Clark (tel: 071-98
6068).**

(4257)A

INSTITUTE OF CANCER RESEARCH
Chester Beatty Laboratories, London SW3
SCIENTIFIC OFFICER AND RESEARCH STUDENTSHIP

1. A Scientific Officer position funded by the MRC AIDS Directed Programme, is available in the Membrane Biology tea
of the Section of Cell and Molecular Biology to undertake wor
on the entry of human immunodeficiency viruses. Applicant
should have a degree in an appropriate biological discipline
and experience in cell biology, immunology or biochemistr
would be an advantage. Salary will be in the range of £12,080-£14,811 pa (inclusive) depending on qualifications and experience. Ref: 7.90.S.N.21.

2. An MRC funded research studentship is available in the Membrane Biology team of the Section of Cell and Molecular Biology. Applicants, with an appropriate degree in the biological sciences, will undertake work on the cellular and molecular analysis of the endocytic pathway, and the role of endocytosis in virus entry. Experience in cell biology, immunology, biochemistry or virology will be an advantage. Ref: 7.90.S.N.22.

To apply, please send a curriculum vitae, in duplicate, to the Personnel Officer, Institute of Cancer Research, 17A Onslow Gardens, London SW7 3AL, quoting the appropriate reference number.

Applicants are advised that smoking is prohibited in the majority of the Institute's premises. (4251)A

UNIVERSITY OF CAMBRIDGE
ARTHUR BALFOUR PROFESSORSHIP OF GENETICS

Applications invited for the above Chair tenable from 1st October, 1991.

Present pensionable stipend £32,408.

Further information from the Secretary General of the Faculties, General Board Office, The Old Schools, Cambridge CB2 1TT, to whom applications (10 copies), marked 'Confidential', should be sent with names of two referees by 14th September, 1990.

The University follows an equal opportunities policy.

(4256)A

Grenoble - Rhône-Alpes - France

The European Synchrotron Radiation Facility is constructing a state of the art storage ring for 6 GeV electrons and/or positrons to be operated as a high brilliance synchrotron radiation source in the field of X-ray from 1994 on.

Financing of the ESRF is shared by 11 European countries.

EUROPEAN SYNCHROTRON RADIATION FACILITY

In the present build up phase, the European Synchrotron Radiation Facility offers you interesting career prospects and a unique opportunity to develop and apply your skills in an international hi-tech environment in fields as varied as

Materials Sciences : diffraction, elastic diffuse scattering, inelastic scattering, extended X-ray absorption fine structure, condensed matter theory, magnetic scattering.

X-ray Beam Lines : design, imaging, optics, detectors.

Physics,

Mechanics,

Ultra High Vacuum,

Power Supplies,

Applied Computer Science : hardware & Software,

Chemistry,

Magnets: undulators & wigglers,

Hydraulies and Cooling,

Radio frequency,

Electronics,

Remote Control & Operation of Complex Machines,

Biology.

We recruit now :

Scientists, Engineers and Technicians*

*Scientists are generally on fixed-term contracts (max. 5 years)

who give evidence of relevant education, and who have experience in research institutes, universities or high-tech industries. Candidates with at least some years of experience in a similar situation, or with a professional background combining education and/or experience from one or more of the fields mentioned above are particularly invited to apply.

The working language is English - knowledge of French is desirable.

For more information about the ESRF, employment conditions and specific vacancies, you can give your name, address and field of professional experience directly to us in Grenoble and we shall provide you with the information needed, and an "Application Form".

ESRF (Recruitment Officer), Boîte Postale 220, F-38043 Grenoble Cedex.

(W7583)A

MEDIA-SYSTEM

Build a Scientific Europe

UNIVERSITY OF GLASGOW

Department of Veterinary Pathology

Technician Grade 3

Applications are invited for the above post funded for 3 years, to work on a study of the role of egg transmitted immunity of salmonellae in the protection of chicks against systemic infection and intestinal colonisation with *S. enteritidis* and *S. typhimurium*. The successful candidate will be expected to work with SPF chickens, to culture salmonellae and aid in the purification of protein antigens. A substantial proportion of the work will involve the separation and assay of antibody from hens and the use and development of ELISA techniques.

Applicants should possess an ONC or equivalent and at least 3 years' relevant experience (including training).

The salary will be within the scale £7,711-£8,645 per annum (under review).

Applications in writing giving details of qualifications and experience should be submitted to the Personnel Officer (Non-Academic), University of Glasgow, Glasgow G12 8QQ not later than 10th August, 1990. Further information can be obtained from Dr. DJ Taylor, Department of Veterinary Pathology, University of Glasgow, Veterinary School, Bearsden Road, Bearsden, Glasgow G61 1QH, tel: 041 330 5700, ext. 5781/6936. (4241)A

United Medical and Dental Schools of Guy's and St Thomas's Hospitals

DEPARTMENT OF PHARMACOLOGY

POSTDOCTORAL RESEARCH ASSISTANT

required on our St Thomas's Campus to study the regulation of calcium channels in heart cells by intracellular messengers, particularly calcium. Techniques include patch clamp and flash-photolysis of 'caged compounds'. Experience with electrophysiological techniques an advantage. Candidates should have a PhD degree. Appointment is for one year in the first instance.

Salary range: £10,458-£16,665 plus £1,767 LWA according to age, and experience. For further information please contact: Dr. A Gurney on 071-928 7739.

Applications with full CV and names and addresses of two referees to the Personnel Officer, UMDS, St Thomas's Campus, Lambeth Palace Road, London SE1 7EH by 9th August, 1990, quoting reference number STH/PHAR/586. (4242)A

DIRECTOR

DEPARTMENT OF TERRESTRIAL MAGNETISM

Carnegie Institution of Washington

The Carnegie Institution of Washington seeks a Director for the Department of Terrestrial Magnetism to lead the Department in a multidiscipinary program directed at fundamental problems in the earth, planetary and astronomical sciences. Applicants should have a record of excellence in research on problems encompassed by this theme. The successful candidate will be expected to carry out an active scientific program and provide general scientific leadership for the staff which currently includes groups in astronomy, geochemistry and seismology. While expertise in each of these disciplines is not required, appreciation of a broad approach to problem-oriented research is essential.

Sitauted in residential surroundings just off Rock Creek Park in Washington, DC. It consists of 14 staff scientists, a like number of post-doctoral associates, and supporting technical staff. Supporting funds are derived from the privately endowed Carnegie Institution with additional income from outside sources. Recent construction and renovation and the relocation of Carnegie's Geophysical Laboratory to the Broad Branch Campus provide excellent surroundings for the future activities of the Department.

Please send a curriculum vitae with cover letter by October 15th to:

Dr Robert Seamans, Jr, Chairman
c/o Ms Susan Vasquez
Carnegie Institution of Washington
1530 P Street, NW
Washington, DC 20005-1910.

EQUAL OPPORTUNITY EMPLOYER

(NW5562)A

MASSEY UNIVERSITY

LECTURER/SENIOR LECTURER IN POST-HARVEST

Seed Technology

The Massey University Seed Technology Centre is seeking to employ a Lecturer or Senior Lecturer to assist with its teaching and research programmes. The successful applicant would be expected to take an active part in undergraduate and postgraduate education and the supervision of overseas students at the Centre. There will be ample scope for the appointee both to develop his or her own teaching and research interests and to participate in existing research and extension activities.

Applicants should have considerable experience in some aspects of post-harvest seed technology and should preferably hold a PhD degree, although those with appropriate work experience will also be considered. Computing and statistical skills would also be desirable.

The appointment will be made initially on a three-year contract.

Enquiries of an academic nature may be made to the Director of the Seed Technology Centre, Massey University, Palmerston North, New Zealand, Fax (64) 63 505-622.

Closing date: 1 October 1990.

Further details of the above position together with Conditions of Appointment are obtainable from Mrs V B Bretherton, Personnel Section, to whom applications, including a full curriculum vitae and the names of three referees should be sent before the closing date specified.

B.R.H. Monks
Registrar

We are an Equal Opportunity Employer (W7590)A

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Telephone (64) 63 69-099

MOLECULAR BIOLOGIST

At the Home Office Central Research and Support Establishment we are looking for an innovative Molecular Biologist to help us break new ground in the field of DNA.

Applications are invited from individuals with either PhD or MSc qualifications to fill a position associated with the:

Isolation and characterisation of genes, such as tyrosinase, which may be associated with commonplace characters such as skin, hair and eye colour. Candidates should have experience of DNA library construction.

The laboratories are fully equipped, including automated sequencing and oligonucleotide synthesiser.

The appointments will be for three years initially with the possibility of an extension to five years. A salary ranging from £14,909 to £21,221 (pay award pending) with benefits such as generous leave allowances, a first-class pension scheme and an excellent working environment make this a unique career advancement opportunity.

If you are suitably experienced for this challenging post, take the first step. Phone Dr Peter Gill on 0734 814100, ext. 2600, for further details and an application form. Interviews are to be held shortly.

The Home Office is an Equal Opportunities Employer and welcomes applications from all suitably qualified persons irrespective of sex, ethnic origin or disability. (4248)A

Leicester University

Department of Biochemistry

POSTDOCTORAL RESEARCH ASSISTANT (IA)

Applications are invited for the post of postdoctoral research assistant tenable for up to two years in the Department of Biochemistry. The project is designed to clone polymorphic loci from canine DNA suitable for use as markers in linkage analysis. Candidates should possess a PhD and, ideally, have a background in genetics and experience of DNA cloning techniques.

Salary will be in the range RA IA (£11,399 to £18,165 pa) depending upon qualifications and experience.

Applications (including CV and names of two referees) should be sent to **Dr J Sampson, Department of Biochemistry, Adrian Building, University of Leicester, University Road, Leicester LE1 7RH.**

Closing date for applications: 10th August, 1990. (4186)A

KING'S COLLEGE LONDON
UNIVERSITY OF LONDON

CHAIR OF FOOD RHEOLOGY

King's College London has a distinguished record of research ar teaching in Food Rheology through the work of Professor P. She man, who retired recently. In recognition of the importance of th area of food research, the College now invites applications for tl newly-established CHAIR OF FOOD RHEOLOGY.

Applicants must be able to demonstrate a strong record of r search in rheology or a closely related area, preferably with e perience of its application to studies of food systems. The appoi tee will be expected to play a leading role in the development of thriving research group in this area, as well as contributing to tl teaching of both undergraduate and advanced level courses. Tl Chair will be held initially within the Food Science Section of tl School of Life, Basic Medical and Health Sciences although ac vities in this area will be restructured in the near future to form new Food Research Group in the School's Division of Biomolec lar Sciences (Biochemistry, Biophysics, Immunology).

Salary will be within the non clinical professorial range.

Further particulars may be obtained from the Deputy Personn Officer, King's College London, Strand, London WC2R 2LS t 071-873 2288.

Applications which should include a curriculum vitae, a descripti of research interests and achievements, a publication list and t names of three referees should be sent to the Deputy Personnel ficer by 17th August, 1990. (4233)A

UNIVERSITY OF CAMBRIDGE
Department of Pharmacology

POSTDOCTORAL RESEARCH ASSOCIATES

Two new posts are available for work with Dr E K Matthews on t following projects:

1. Dynamic laser - light scattering spectroscopy of single secreto cells: funded by the Wellcome Trust.
2. Photodynamic drug action on the exocrine pancreas: funded the CRC.

The Department is located in new purpose-built laboratories in t centre of Cambridge with excellent facilities for research.

Each appointment will be for up to three years with salary in the ran £10,458-£15,372 (currently under review).

Applications, including CV and names of two referees, to: **Dr E Matthews, Department of Pharmacology, University of Ca bridge, Tennis Court Road, Cambridge CB2 1QJ, UK. Tel: (022 334032.** (4229)

UNIVERSITY OF EDINBURGH

CRC Laboratories, epartment of Pathology

RESEARCH ELLOW/ASSOCIATE IN ANCER RESEARCH

plications are invited for a Re- rch Fellow/Associate (IA or IB) be based in the Cancer Re- rch Campaign Laboratories in Department of Pathology at University of Edinburgh for a iod of 3 years commencing ptember/October 1990. The rk which will be in conjunction the University Department of rmatology, will be concerned h the role of human papilloma uses in the development of cu- eous and genital cancers in im- nosuppressed patients.

plicants should have experi- ce of molecular biology and/or l biology. Appointments will be ade on the RA/IA (currently 1,399-£18,165) RA/IB (£11,399- 3,495) scales, according to alifications and experience.

rther information and arrange- ents to visit the Departments can made with Professor C C Bird el 031-667 1011 ext 2227).

plications with CV and names two referees should be sent to ofessor C C Bird, Department Pathology, University Medical hool, Teviot Place, Edinburgh 18 9AG by 17th August 1990.

ease quote ref. 5792.

(4231)A

UNIVERSITY OF NOTTINGHAM

Department of Genetics

POSTDOCTORAL RESEARCH ASSISTANT

pplications are invited for an RC-funded position to work ith Dr. G.T. Morgan, for one ear in the first instance, on the ontrol of transcription termi- ation in *Xenopus*. Experience molecular biology and DNA anipulation is desirable. alary will be on the Research A scale.

Applications, including a *cur- iculum vitae* and the names of wo referees should be sent as oon as possible to Dr. Garry organ, Department of Gene- cs, University of Nottingham, ueens Medical Centre, Not- ingham NG7 2UH, tel: 0602 09402.

(4238)A

OPENING FOR POSTDOCTORAL

to study molecular details of import and structure-function of colicin E1 channel, combining approaches of membrane protein biochemistry-biophysics and site-directed mutagenesis (see *JBC. 264,* 1959; *Molec. Microbiol., 4,* 519; *Biochem., 29,* 5829).

Submit cv, statement of research interests, and names of 3 referees to W A Cramer, Dept of Biological Sciences, Purdue University, West Lafayette, IN, 47907, USA.

Equal opportunity/affirmative action employer. (NW5568)A

**The Department of Health
in collaboration with the
Health and Safety Executive
is calling for research proposals in**

HSE
Health & Safety Executive

Childhood Leukaemogenesis

In their Second Report, the independent expert advisory Committee on Medical Aspects of Radiation in the Environment (COMARE) recommended further laboratory research to assess the importance of preconception, fetal and early childhood exposure to ionizing radiation in leukaemogenesis. Furthermore, COMARE, in their Statement of Advice to Government*, outlined several possible causative mechanisms (not necessarily involving ionizing radiation) which might explain the documented statistical association between the excess of leukaemia and non-Hodgkin's lymphoma in the vicinity of Sellafield and employment of the fathers of the affected children at the plant. The Committee stated that further biological research was required to investigate these possible causative mechanisms.

Applications are invited from researchers at Universities and equivalent research units in the UK. For further information and application forms (MRC style) please write to: Anthony Cummins, EHF Division, Department of Health, Room 918, Hannibal House, Elephant and Castle, London SE1 6TE, enclosing a large self-addressed envelope. Please mark your envelope "CCHARR".

*Hansard 2 April Vol 167 Cols 430-434.

The Department of Health and the Health and Safety Executive are Equal Opportunities Employers

(4244)A

The Department of Molecular Biology at Princeton University

invites applications for a

junior level position from investigators who use the mouse as an experimental system. Areas of specialization may include (but are not restricted to) immunology, genetics, neurobiology, developmental biology, chromosome structure, or genome analysis. Candidates should have a Ph.D degree and appropriate experience in mouse molecular biology. Applicants should send a curriculum vitae, a summary of research interests, and the names of at least three referees to:

**Mouse Molecular Biology Search Committee
Department of Molecular Biology
Princeton University
Princeton, New Jersey 08544**

Application deadline is 20th October, 1990.

Princeton University is an Equal Opportunity/Affirmative Action Employer. (NW5564)A

POSTDOCTORAL POSITION

Position available for an individual with a Ph.D. and experience in molecular biology. The successful applicant will be engaged in research on the biochemical and molecular mechanisms by which angiotensin peptides and other hormonal and pharmacological stimuli mediate cardiac muscle cell growth. There will also be the opportunity to engage in research defining the role of a localized intracardiac reninangiotensin system in mediating cardiac muscle cell growth, with an emphasis on the regulation of the precursor genes for this system. Competitive salary and fringe benefits are available.

Please send CV and three names or letters of reference to: **Dr. Kenneth M. Baker, Weis Center for Research, Geisinger Clinic, Danville, PA 17822.**

Equal opportunity employer.

(NW5558)A

THE UNIVERSITY OF MANCHESTER

CHAIR OF CANCER EPIDEMIOLOGY

The University invites applications for a Chair of Cancer Epidemiology, following the retirement of Professor Alwyn Smith. Preference may be given to candidates with medical qualifications registrable in the United Kingdom. The appointee will be employed within the University's Department of Public Health and Epidemiology, and will also be a member of the multi-disciplinary Group at the Christie Hospital and Holt Radium Institute and the Paterson Institute for Cancer Research.

Applications (one copy suitable for photographic reproduction), giving full details of qualifications and experience, together with the names and addresses of three persons to whom reference may be made, should be sent, not later than 1st October, 1990, to the **Registrar, the University, Manchester M13 9PL, from whom further particulars may be obtained, tel: 061 275 2028. Quote ref. 219/90/NA. Overseas candidates may apply by facsimile tel: 061 275 5584 in the first instance.**

The University is an Equal Opportunity Employer. (4239)A

UNIVERSITY OF READING

DEPARTMENT OF BIOCHEMISTRY & PHYSIOLOGY

RESEARCH OFFICER

Applications are invited from graduates in the biological sciences to work as a Research Officer on an MRC-funded project 'Endocrine and paracrine aspects of corticotrophin releasing hormone in hypertension and other abnormal pregnancy'. This involves placental tissue culture and immunoassay of stress hormones, for which training will be given. The successful applicant may register for a PhD. The post is for 3 years from 1st October. Starting salary approx. £11,399 p.a. Informal enquiries to Dr. E.A. Linton on (0734) 875123 ext. 7048. *Apply for Application Form* to Personnel Officer, University of Reading, Whiteknights, P.O. Box 217, Reading RG6 2AH, telephone (0734) 318754. Please quote ref. R9028.

(4236)A

THE AUSTRALIAN NATIONAL UNIVERSITY

Applications are invited for appointment to the following positions:

RESEARCH SCHOOL OF PHYSICAL SCIENCES

RESEARCH FELLOW/POSTDOCTORAL FELLOW IN ULTRAVIOLET PHYSICS

Atomic and Molecular Physics Laboratories

the Atomic and Molecular Physics Laboratories (Head: Professor R W Crompton FAA) is seeking to appoint a research physicist in the Ultraviolet Physics Unit. The Unit undertakes experimental and numerical studies on problems of atmospheric, aeronomic and astrophysical significance. Photoabsorption and fluorescence spectroscopy is performed using conventional VUV sources and ultra-stable 2.2m scanning monocromator, and, in a joint facility with the Laser Physics Centre, a variety of excimer and dye lasers enable the generation of coherent VUV radiation for ultra-high resolution measurements. The program of numerical molecular physics is, at present, concentrated on the treatment of interactions between molecular electronic states through the numerical solution of multiple-coupled Schrodinger equations. The successful applicant will hold a PhD in Physics and should have appropriate postdoctoral experience. Expertise in both experimental (using conventional and/or coherent laser VUV sources) and computational molecular physics would be a definite advantage. Enquiries may be directed to Dr B R Lewis (tel. (61 6) 249 2460).

Closing date: 30th August 1990 *Ref: PS 17.5.1*

RESEARCH FELLOWS/ POSTDOCTORAL FELLOWS

Department of Nuclear Physics

The Department (Head: Dr T R Ophel) conducts research in nuclear structure physics. The purpose of the work is to gain greater understanding of both the static and dynamic aspects of the isolated atomic nucleus and of its interactions with other nuclei when collisions occur. Currently, the principal areas of research are: reactions with heavy ions, including resonant structure; measurements of static quadrupole moments of excited states through Coulomb excitation; nuclei with very high angular momenta studied through gamme, particle and fission decay; production of exotic nuclei far from the stability line. A very active accelerator mass spectrometry program haa been established using the 14UD accelerator. Though comprising mainly of measurements of chlorine-36, some carbon-14 measurements are made and development of techniques for other radio-isotopes is in progress. No teaching is required.

Closing date: 30th September 1990. *Ref: PS 17.5.2*

RESEARCH FELLOW/ POSTDOCTORAL FELLOW

Department of Theoretical Physics

The Department (Head: Dr B A Robson) is engaged in research into theoretical aspects of atomic and molecular physics, condensed matter physics, non-linear dynamics, nuclear physics, particles and fields, plasma physics, quantum mechanics and statistical mechanics. In this instance, preference will be given to candidates in the field of atomic and molecular physics with special interests in *ab initio* calculations of electron scattering and photon absorption from atoms and molecules.

Closing date: 30th August 1990 *Ref: Ps 17.5.3*

SALARY: Research Fellow A$32,197-A$41,841 pa; Postdoctoral Fellow Grade 1 (fixed point) A$27,953-A$31,808 pa. *APPOINTMENT:* Research Fellow up to three years, possibility of extension to five years; postdoctoral Fellow up to two years, possibility of extension to three years. *APPLICATIONS* should be submitted in duplicate to the Registrar, The Australian National University, GPO Box 4, Canberra ACT 2601, Australia, quoting reference number and including curriculum vitae, list of publications and the names and addresses of at least three referees. The University reserves the right not to make an appointment or to appoint by invitation at any time. Further information is available from the Registrar or from the Appointments Officer, Association of Commonwealth Universities, 36 Gordon Square, London WC1H 0PF.

THE UNIVERSITY IS AN EQUAL OPPORTUNITY EMPLOYER

(W7592)A

MOREDUN RESEARCH INSTITUTE

VACANCY IN MOLECULAR IMMUNOLOGY

(4-year appointment)

An opportunity exists for a molecular immunologist, immunologis or molecular biologist to join a multidisciplinary team which i carrying out molecular studies of the class II region of the ovin major histocompatibility complex. The appointee will study th expression, subtype distribution and function of class II genes *i vitro* and *in vivo* using immunological and genetic techniques. Th work will integrate with that of both a linked research programm with the Royal (Dick) Veterinary School at Edinburgh Universit and the Department of Immunology at the Institute for Anima Physiology and Genetics Research, Babraham, England.

Qualifications: First or upper second class honours degree o equivalent in appropriate scientific subject. PhD preferred with a least 2 years relevant postgraduate research or other experience

Salary: HSO Scale (currently £10,678-£14,909).

Further details available from Mrs I. Ritson, Personnel Office Moredun Research Institute, 408 Gilmerton Road, Edinburgh EH17 7JH, to whom applications, including the names of tw referees should be submitted.

Ref: MR1/2.

Closing date: 10th August, 1990. (4235)A

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(4285)A

dkfz

The Deutsche Krebsforschungszentrum (German Cancer Research Center) offers a position as

Head of a new Group for

Molecular Pharmacology

We wish to appoint a young qualified scientist with excellent records in molecular pharmacology related to oncology.

The applicant should have qualifications comparable to a German "Habilitation" (Assistant Professorship).

The position is initially limited to a 5 years period with a salary in the range of approximately DM 80,000 per annum. It offers applicants the opportunity for research in a well equipped laboratory. Positions for two postdocs and two technicians will be available.

Applicants should submit their curriculum vitae together with a list of publications and references by August 31st, 1990 to Prof H zur Hausen, Wissenschaftlicher Stiftungsvorstand des Deutschen Krebsforschungszentrums, Im Neuenheimer Feld 280, D 6900 Heidelberg, Germany. (W7588)A

LONDON SCHOOL OF HYGIENE & TROPICAL MEDICINE

IMMUNOBIOLOGY OF PARASITIC DISEASES UNIT

Position 1: Senior Research Fellow RII/RIII
(salary range: £19,613-£21,134+£1,767 London Weighting)
Position 2: Research Fellow RIa
(salary range: £17,455-£18,888+£1,767 London Weighting)

Applications are invited for two positions for mammalian molecular geneticists to work on Dr. Jenefer Blackwell's Wellcome Trust funded programme to clone the disease resistance gene *Lsh/Ity/Bcg* on mouse chromosome 1. The project will involve the use of chromosome microdissection and YAC cloning techniques. Applicants should have a sound training in molecular genetics and a broad interest in infectious disease research.

Applications in the form of a current curriculum vitae including the names and addresses of at least two academic referees should be forwarded as soon as possible to the Personnel Officer, LSHTM, Keppel Street, London WC1E 7HT (tel: 071-636 8636 ext. 203) quoting reference JB1. Informal enquiries may be made to Dr. Jenefer Blackwell on 071-636 8636 ext. 217.

Closing date: 30th September, 1990. (4243)A

BOSTON UNIVERSITY MEDICAL CENTER
EVANS MEDICAL RESEARCH DEPARTMENT

DIRECTOR

NEW RESEARCH TRAINING PROGRAM

The Evans, one of the nation's oldest endowed basic and clinical research departments, seeks a director for an innovative research training program. The program will enhance the already excellent environment for post-doctoral/fellowship training of both Ph.D. and M.D. scientists. Endowment funds are available to support trainees and an enriched program of visiting senior scientists, seminars, methods courses, etc.

The Evans staff currently includes about 100 basic scientists and clinical investigators and 60 post-doctoral students/fellows. Over 75,000 square feet of modern research space is now in use and expansion is planned.

The Director of the new research training program should be a scientist of recognised achievement interested in training of basic and clinical investigators for interactive medical research. Both senior basic/clinical scientists (including distinguished emeritus researchers) and established younger scientists will be considered. Funds and space are available for Director's personal research. Reply to: **Dr. Norman G. Levinsky, Director, Evans Department, Boston University Medical Center, Boston, MA02118.** (NW5559)A

UCMSM

UNIVERSITY COLLEGE & MIDDLESEX SCHOOL OF MEDICINE

Professor and Head of the Department of Medicine

Applications are invited for an established Chair of Medicine with the appointee assuming the headship of the Department of Medicine on the retirement of Professor S J Semple in September 1991.

Applicants should have a strong commitment to undergraduate and postgraduate teaching and a personal record of distinction in research. The Department of Medicine has academic activities in association with The Middlesex, University College and Whittington Hospitals; no specific specialty interest of applicants is being sought.

Applications including a full curriculum vitae and the names of three referees should be sent by 23rd August, 1990 to the Provost, University College London, Gower Street, London WC1E 8BT. Further particulars about the post are available from the Dean, University College and Middlesex School of Medicine, Riding House Street, London W1P 7PN (Tel: 071-380 9306).

Equal Opportunities Employer.

UCL

(4264)A

DEPARTMENT OF HUMAN ANATOMY AND CELL BIOLOGY

LECTURER – ANATOMY OR ANATOMY/PRIMATOLOGY

Initial salary within the range £10,458 - £14,169 p.a. (under review)

Candidates will be expected to contribute to the teaching of human morphology and, if suitably qualified, to the teaching of primatology. In addition, candidates will be expected to develop a programme of research that strengthens, or complements, the Department's present interests in Cell Biology, Neuroscience and Primatology. Excellent facilities exist for 3D-image analysis, microscopy, morphometry and cell biology.

Informal enquiries to Professor Bernard Wood on 051-794 5494.

Quote ref: RV/773/NAT

Closing date: 10 August, 1990

DEPARTMENT OF BIOCHEMISTRY

POSTDOCTORAL SENIOR RESEARCH ASSISTANT

Initial salary within the range £10,458 - £12,879 p.a. (under review)

Tenable for up to eighteen months.

Supported by the SERC and English Heritage to join a group investigating preserved organic matter of archaeological interest.

The work is well-funded and of a multidisciplinary nature. Experience in the chromatographic and mass spectrometric analysis of natural products would be desirable, although not essential, as training would be available in these areas.

Informal enquiries to Dr. R.P Evershed on 051-794 4345 or 051-794 4348.

Quote ref. RV/767/NAT

Closing date: 9 August, 1990

Applications, by c.v. with the names of three referees, should be received by the Director of Staffing Services (AS), The University, P.O. Box 147, Liverpool L69 3BX, from whom further particulars may be obtained. (4268)A

University of LIVERPOOL

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UNIVERSITY OF CAMBRIDGE

PROFESSORSHIP OF BOTANY

Applications invited for the above Chair tenable from 1st October, 1991.

Present pensionable stipend £32,408.

Further information from the Secretary General of the Faculties, General Board Office, The Old Schools, Cambridge CB2 1TT, to whom applications (10 copies), marked 'Confidential', should be sent with names of two referees by 14th September, 1990.

The University follows an equal opportunities policy.

(4255)A

THE UNIVERSITY OF LEEDS

LEUKAEMIA RESEARCH FUND
CENTRE FOR CLINICAL
EPIDEMIOLOGY

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RESEARCH
FUND
Patron of the Fund :
HRH The Duke of Kent

EPIDEMIOLOGIST

This exciting post is in a leading research group investigating aspects of the epidemiology of leukaemi and lymphomas. It is anticipated that the successful candidate will develop major interests in childhood leukaemias and be responsible for a childhood tumour registry. In addition new large case-control studies are being planned and it is anticipated that the appointee will have a major role to play in their conduct.

The position is available at present until the end of 1991, in common with all others in the Centre, with a strong chance of future funding.

Candidates should hold a PhD or have equivalent experience in an area related to epidemiology.

Salary on the scale for Research Staff Grade IA (£10,458-£16,665) (pay award pending).

Further details and application forms can be obtained from, and completed applications returned to, the Registrar, The University, Leeds LS2 9JT (Tel: 0532 333969—direct line), quoting reference number 102/48. Closing date for applications: 14th August, 1990.

The University of Leeds is an equal opportunities employer.

(4265)A

FACULTY POSITIONS

Molecular Evolutionary Genetics — Penn State

The department of Biology, in association with its new Institute of Molecular Evolutionary Genetics, seeks to fill several tenure-track positions in evolutionary genetics, at the assistant or associate professor level. Research areas of interest are the population genetics of molecular variation, the evolution of genes and regulatory systems, and the application of molecular techniques to the study of morphological evolution and biosystematics. Significant research accomplishments and evidence of potential to develop an outstanding research program will be key considerations.

Applicants must have post-doctoral training.

Send curriculum vitae, statement of research interests, and three letters of reference to: Dr Robert K Selander, Chairman, Search Committee, Department of Biology and Institute of Molecular Evolutionary Genetics, Box A1, 208 Mueller Laboratory, Penn State University, University Park, PA 16802. Application deadline: August 26, 1990.

An Affirmative Action/Equal Opportunity Employer. Women and Minorities Encouraged to Apply. (NW5553)A

UNIVERSITY OF NOTTINGHAM

Department of Pharmaceutical Sciences

LECTURESHIP IN DRUG DELIVERY

Applications are invited from suitably qualified candidates for a Lectureship in Drug Delivery. The successful candidate will join a very active group working on various aspects of drug delivery to include controlled release systems, drug targeting and advanced delivery systems for genetically engineered drugs.

Salary within the range £10,458-£20,469 per annum (under review) depending on age and experience.

Further details and application forms, returnable not later than 24th August 1990, from the Personnel Office, University of Nottingham, Nottingham NG7 2RD (tel 0602 484848 ext 3355). Ref No 1357. (4262)A

The Department of Molecular Biology at Princeton University

invites applications for a

tenure track position at the Assistant Professor level in the area of Developmental Neurobiology. We are especially interested in individuals who are working on the development or functioning of the nervous system in organisms which are amenable to both genetic and molecular analysis. Applicants should send a curriculum vitae, a summary of research interests, and the names of at least three referees to:

Developmental Genetics and Neurobiology Search Committee
Department of Molecular Biology
Princeton University
Princeton, New Jersey 08544

Application deadline is 20th October, 1990.

Princeton University is an Equal Opportunity/Affirmative Action Employer. (NW5563)A

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Virologist/Projectleader

for our virological research and development department

Akzo is one of the largest chemical concerns in the world with headquarters in Arnhem, 350 branches in 50 countries and more than 70.000 employees. A job with Akzo means a career at a technologically advanced company, active in the fields of chemistry and chemical fibres, paints and varnishes and health care products.

Intervet International bv - a dynamic, fast-growing company and part of Akzo's Pharma Division - develops, produces and sells veterinary medicines. The Intervet group has some twenty establishments throughout the world and a total of 1100 employees. The company's headquarters in Boxmeer employ 450 people, more than 100 of which work in R&D. Products are exported worldwide.

The Virological R&D department, develops and improves vaccines for combatting viral infections in live stock and pet animals.
All available techniques are used, from genetic engineering to classical virological procedures. Work on the various topics is carried out projectwise by a multi-disciplinary team working inside and occasionally, outside Intervet. The projectleader maintains relations with the scientific community. After an initial period, the appointee is expected to manage a project team which directs the development of a vaccine from start to finish.

Applicants should be approximately thirty years old and have an academic degree in one of the following subjects: biology, biochemistry, biotechnology or veterinary medicine and are experienced in cell culture and virological techniques.

For more information please contact Dr. N. Visser, telephone ++31.8855-87354 (work) or 31.8855-75878 (private).
Applications should be directed to Personnel Department, Intervet International bv, att. mr. H. Verbeek, Postbus 31, 5830AA Boxmeer, the Netherlands.

AKZO

(W7581)A

The University of Texas Health Center at San Antonio

PROFESSOR AND CHAIR

Department of Physiology

Applications and nominations for the position of Professor and Chair of the Department of Physiology at The University of Texas Health Science Center at San Antonio are invited. The Department has 19 full time faculty with diverse research interests including cellular, cardiovascular, respiratory, and neurophysiology, and the metabolic aspects of ageing. The Department has a well supported graduate program and has instructional responsibilities in the Graduate, Medical, Dental, and Nursing Schools. Candidates should have an active research program in a defined area of contemporary physiology ranging from the molecular to the intact animal. Individuals should possess an internationally recognised record of research with experience in teaching and administration. Interested persons should send a letter of application, curriculum vitae, a brief statement of research interests, and names of three references. The committee will begin reviewing applications by October 1st, 1990. Address applications, inquiries and/or nominations to: **David S. Papermaster, M.D., Physiology Chair Search Committee, Department of Pathology, The University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Drive, San Antonio, TX 78284-7750.**

UTHSCSA is an Equal Opportunity/Affirmative Action Employer. (NW5555)A

CHIEF

SPECIAL PATHOGENS BRANCH

Division of Viral and Rickettsial Diseases, Center for Infectious Diseases, Centers for Disease Control

The Centers for Disease Control is seeking qualified applicants for the position of Chief, Special Pathogens Branch, Division of Viral and Rickettsial Diseases, Center for Infectious Diseases. The Branch Chief directs and participates in comprehensive epidemiologic, clinical, and laboratory programs concerning the biology, molecular biology, immunology, diagnosis, prevention, and treatment of diseases caused by unique viral pathogens such as filoviruses (eg Marburg virus, Ebola virus), arenaviruses (eg Lassa fever virus), and bunyaviruses (eg Crimean-Congo hemorrhagic fever virus, Hantavirus) in the United States and abroad. The Branch is housed in a new research facility that contains 7,000 square feet of state-of-the-art biosafety level 3 and 4 laboratories.

Qualifications preferred include a doctoral degree (MD, PhD, or equivalent), major scientific accomplishments in one or more areas of relevant research, and the ability to provide administrative and scientific leadership for a multidisciplinary program employing 20 scientific and support staff.

Salary range is approximately $59,000 to $76,000 per annum, depending on qualifications.

Further information about the position can be obtained from Larry J Anderson, MD, Chairman, Special Pathogens Branch Chief Search Committee, Division of Viral and Rickettsial Diseases, Bldg 7 Rm B43 (G17), Centers for Disease Control, Atlanta, Georgia 30333.

Interested candidates may send their curriculum vitae in confidence along with the names of three professional references by September 15th, 1990 to: Sharlyn L Wilkinson, Personnel Management Office, 1-1058, Centers for Disease Control, 1600 Clifton Road, NE (D01), Atlanta, Georgia 30333.

CDC is an Equal Opportunity Employer and provides a smoke-free work environment. (NW5560)A

APPLICATION SCIENTIST/ COMPUTATIONAL CHEMIST

BIOSTRUCTURE, the European leader in Molecular Modelling Systems, is growing.

Immediate openings are available for outstanding candidates.

The successful PhD scientist(s) will have expertise in the Molecular Modelling field, related to one of the following areas:

- NMR spectroscopy,
- X-ray crystallography,
- Molecular engineering,
- Drug design,
- Polymer design.

The position requires excellent communication skills. You will join a team of scientists designing tomorrow's Molecular Modelling products. Familiarity with modelling tools (Molecular Mechanics and Molecular Dynamics technics) and computer systems is recommended.

Please forward your curriculum vitae and names of two references to:

Mr Jean Marie Wurtz
Biostructure S A
8 Rue Gustave Adolphe Hirn
67008 Strasbourg, France

(W7594)A

PENNINGTON BIOMEDICAL RESEARCH CENTER

ANNOUNCES

Openings for Molecular Nutrition Research Program. The Pennington Biomedical Research Center is a 223,000 square feet research facility opened in 1987 and located near the LSU campus in Baton Rouge, Louisiana. Positions include the following scientists:

1. Program Chief/Professor, a tenure track appointment in LSU and holder of the Claude Bernard Chair which has a $1 million endowment at the Center. The Program Chief will assemble and direct the scientific teams in Molecular Nutrition research. 10,000 square feet of wet lab space are currently available in our state-of-the-art facility for development of this program. There is a $600,000 allowance for initial equipment purchases. Dedicated salary funding is allocated to recruit a team of additional scientists, fellows and technicians.

2. Professor

3. Associate Professor

4. Assistant Professor

A Ph.D. or M.D. and appropriate research experience in molecular biology and nutrition research are necessary. Expertise in aflatoxins, mycotoxins, or carcinogenesis research desirable for one of the tenured positions. Appropriate publication record required. Demonstrated ability to procure funding necessary. Qualified candidates should send their application by August 1st, 1990 to:

Dr. George Bray, Executive Director, Pennington Biomedical Research Center, 6400 Perkins Road, Baton Rouge, LA 70808.

LSUMC is an EEO/AA Employer.

(NW5557)A

PHILIPPS-UNIVERSITAT
MARBURG

NEUROBIOLOGY GROUP
Department of Anatomy and Cell Biology
University of Marburg, Germany

MOLECULAR BIOLOGISTS/PROTEIN BIOCHEMISTS

sought at *early and senior postdoc levels* for projects related to growth factor expression, regulation and functions in the nervous system focusing on the FGF and TGF-ß families. Positions are available for two to five years with the prospect of prolongation. Salary according to experience and qualifications.

Send CV and names of two referees to Prof K Unsicker, Dept Anatomy and Cell Biology, University of Marburg, Robert-Koch-Str 6, D-3550 Marburg, FRG (tel.-6421-284030 or fax -6421-285783). (W7589)A

HEAD — Department of Genetics

The College of Agriculture and Life Sciences of North Carolina State University invites nominations and applications for the position of Head of the Department of Genetics. Duties will be to provide leadership and administer a strong department of 19 faculty members, and to teach or conduct research in some area of genetics. Candidates should have an established scientific reputation in genetics, a PhD or equivalent degree and administrative experience or demonstrated potential for leadership.

Applications should include a curriculum vitae, names and addresses of at least three references, and a statement describing applicant's interest and qualifications for the position. Screening of applications will begin 15th September, 1990 and continue until a suitable candidate is found.

Address application and nominations to Dr E D Seneca, Chairman, Search Committee, Botany Department, Box 7612, North Carolina State University, Raleigh, NC 27695-7612, USA.

North Carolina State University
College of Agriculture and Life Sciences

North Carolina State University is an Equal Opportunity/Affirmative Action Employer. (NW5561)A

DIRECTOR

Marine Biomedicine

The University of California, San Diego invites applications from scientists with an oustanding record in research and teaching for the position of Director of Marine Biomedicine, jointly sponsored by the School of Medicine (SOM) and Scripps Institution of Oceanography (SIO). Applicants must qualify for a full professorial appointment (tenured) in an appropriate Department, should possess interests relevant to the SOM and SIO, and should have leadership qualities suitable for directing a newly developing effort in marine biomedicine, a broad-based academic activity. The Physiological Research Laboratory, located on the SIO campus, composed of faculty, staff, and students from both the SOM and SIO, will remain one component of efforts to foster substantial growth in marine biomedicine at UCSD. It is anticipated that the expanded program will involve both areas of current collaboration between SIO and SOM, such as neurobiology, biomarine chemistry, and marine pharmacology.

Salary commensurate with qualifications and based on established UCSD salary schedule.

Send letter of application, CV, reprints and names and addresses of referees by October 1st, 1990 to: **Search Committee Co-Chair, Frank Powell, PhD, Associate Professor, Department of Medicine, Division of Physiology, M-023A, University of California, San Diego, La Jolla, CA 92093.**

An Equal Opportunity/Affirmative Action Employer. (NW5550)A

UCLA NEUROSCIENCE

DIRECTOR

UCLA JERRY LEWIS NEUROMUSCULAR RESEARCH CENTER

The UCLA School of Medicine is recruiting a new Director for its Jerry Lewis Neuromuscular Research Center. The individual is expected to bring leadership in new areas of science fundamental to understanding broad aspects of nerve and muscle and the disease processes that affect them. Programs focussed in molecular biology, immunology or genetics with particular strengths relevant to understanding clinical neuromuscular diseases would be particularly attractive.

The Director will assume responsibility for the Jerry Lewis Center, an organized research unit on the UCLA campus reporting directly to the Dean's office, with the members having appointments in one or more School of Medicine departments. This individual will be expected to promote the development of basic and clinical research programs and facilitate teaching and training in neuromuscular biology and disease.

Interested applicants should forward current curriculum vitae to Robert C Collins, MD, Chair, Search Committee, UCLA School of Medicine, Reed Neurological Research Center, 710 Westwood Plaza, Los Angeles, CA 90024-1769.

UCLA is an equal opportunity/affirmative action employer. (NW5574)A

THE UPJOHN COMPANY

Director, Cell Biology

The Upjohn Laboratories Division of The Upjohn Company, a worldwide leader in pharmaceutical research, is seeking a Director to lead our Cell Biology Research Unit as well as maintain their own active research effort. The successful candidate will be responsible for directing a multi-disciplinary research group of 30-40 scientists and staff working in several areas of cellular and molecular biology including protein export, cellular immunology, receptor biology and second messenger systems, and growth control. The focus of the department is to use cellular approaches to complement and support drug discovery programs at Upjohn.

Qualified applicants will possess:

- **A Ph.D. and/or M.D. with an established record of scientific excellence as evidenced by publications and/or patents.**
- **Commitment to drug discovery and development.**
- **Administrative experience and excellent communications skills.**

Upjohn offers a competitive compensation and benefits package in addition to a stimulating, mult-idisciplinary research environment at our modern laboratories in Kalamazoo, Michigan. In 1989, Upjohn committed over $400 million to research and development and is currently increasing its main research facility by 50%. Qualified and interested applicants are invited to forward their cv/resume to: THE UPJOHN COMPANY, CORPORATE RECRUITING , KALAMAZOO, MI 49001-0199. Refer to position number 5697-HA in your cover letter. An Equal Opportunity Employer M/F.

Our Commitment to Scientific Excellence Continues.... (NW5567)A

Upjohn

UNIVERSITY OF NOTTINGHAM

LECTURESHIP IN PLANT MOLECULAR BIOLOGY

Applications are invited for a new Lectureship in Plant Molecular Biology in the Department of Botany. Candidates should have experience in studying plant systems and their physiological development using molecular, biochemical and physiological techniques. In addition to participating in undergraduate teaching the successful candidate will be expected to develop a program of research which complements that of the Plant Genetic Manipulation Research Group. Excellent facilities for research in this area already exists.

Further information may be obtained informally from Professor Edward Cocking, FRS (Telephone 0602 484848 ext 2201; Fax 0602 424270). Salary will be on the Lecturer A Scale £9,816-£15,372 per annum and the post is available from 1st October 1990.

Further details and application forms, returnable not later than 28th August, from the Personnel Officer, University of Nottingham, Nottingham NG7 2RD (Telephone 0602 484848 ext 3355) reference number 1356. (4261)A

CAMBRIDGE LABORATORY

John Innes Centre for Plant Science Research

MOLECULAR PLANT PATHOLOGIST

Applications are invited for a research position (Higher Scientific Officer) in the well-equipped Cereals Research Department at the Cambridge Laboratory, which is now located at Norwich, to investigate the organisation of the complex Mla locus in barley. Mla includes numerous different alleles for resistance to powdery mildew. The work will use novel screening methods, YAC libraries and doubled-haploid lines to map DNA sequences close to MLA. The successful applicant will be assisted by a plant pathologist and will be able to cooperate with other JICPSR programmes on barley genetics, RFLP mapping in cereals, molecular genetics and plant pathology. The post is available for three years.

Applicants should have a first or upper second class honours degree in Molecular Genetics, Plant Pathology or a similar subject with at least three years' relevant post-graduate experience. The salary will be on the scale: £10,678-£14,909 p.a. (starting point subject to qualifications and experience). Pay award pending. A non-contributory Superannuation Scheme is in operation.

Applications should be made to: The Personnel Officer, John Innes Institute, Colney Lane, Norwich NR4 7UH quoting reference PG111/559 and including a curriculum vitae and the names of three referees, by 17th August, 1990. Equal Opportunities Employer. (4249)A

UNIVERSITY OF MANCHESTER

GRADUATE RESEARCH ASSISTANT

Experimental Models of Parkinsonism

Applications are invited, from graduates in any branch of neuroscience, for this post in the Experimental Neurology and Myology Group, Department of Cell and Structural Biology. The post is funded for 2 years by the Wellcome Trust and will investigate the neural mechanisms which mediate experimental parkinsonism. Behavioural, anatomical and pharmacological approaches will be used.

Appointment will be for one year in the first instance, with a salary of £10,458 pa on Grade 1B (under review).

Applications including curriculum vitae and the names of two referees should be sent by 15th August to Dr I J Mitchell, Department of Cell and Structural Biology, University of Manchester M13 9PT, UK. Tel: 061 275 5262/5282.

THE UNIVERSITY IS AN EQUAL OPPORTUNITY EMPLOYER (4260)A

UNIVERSITY OF OTTAWA

Department of Biology

POSTDOCTORAL RESEARCH ASSOCIATE

Position available immediately to join a university-industry group working on the immunodiagnosis and biological control of sapstain fungi. Training in immunochemistry/biochemistry is preferred. Salary up to $30,000.00/yr depending on experience.

Applicants should submit curriculum vitae with names and telephone numbers or referees to: **Dr David L Brown, Department of Biology, University of Ottawa, Ottawa, Canada K1N 0NS or Fax 613-564-5014.** (NW5572)A

University College London
Department of Physiology
POST-DOCTORAL RESEARCH ASSISTANT
(Molecular Biology)

to join new group investigating gene regulation in the annexin family of calcium-binding proteins. Some experience in molecular biology desirable but not essential. The interests of the laboratory are protein phosphorylation (two of the annexins are targets for phosphorylation by transforming and signal transducing tyrosine kinases), and developmental and functional regulation of expression. The project involves the characterization and functional analysis of the gene for one of these proteins. Some of the work will be done in collaboration with Dr Michael J. Crumpton at the Imperial Cancer Research Fund.

The post is funded by a grant from the Wellcome Trust for a period of three years, and is available from 1st October 1990. The salary is on the Research Assistant Scale 1A, starting between £16,000-£17,500 per annum depending on age and experience.

Apply in writing (enclosing two copies of a CV) to Dr Stephen E Moss at the Department of Physiology, University College London, Gower Street, London WC1E 6BT, UK.

EQUAL OPPORTUNITIES EMPLOYER

(4274)A

ANIMAL DEVELOPMENTAL BIOLOGY
Cornell University
College of Veterinary Medicine

Applicants are sought for TWO faculty positions (Assistant/Associate Professor) to be part of a campus-wide initiative in Animal Molecular Biology. Individuals in the following areas are invited to apply: Embryo Biology (transgenic animal studies, embryonic stem cells, embryo manipulation)l Molecular Genetics (gene regulation in early development, growth factors and oncogenes, genome mapping); and Animal Reproduction (molecular endocrinology, genetic imprinting).

The College of Veterinary Medicine at Cornell is undertaking substantial expansion that will permit advanced studies of a wide variety of mammalian and avian species. The Animal Developmental Biology Program will complement existing strengths in reproduction in the Veterinary College, and excellent University programs in basic biology, animal science, genetics and biotechnology. The successful candidates will hold faculty appointments in appropriate departments of the Veterinary College and will participate in its graduate and professional teaching programs. Veterinary qualifications and/or experience with domestic animals are desirable, but not required.

Send a letter of interest with a resume and the names of 3 references to: **Dr Douglas Antczak, James A Baker Institute for Animal Health, New York State College of Veterinary Medicine, Cornell University, Ithaca, New York, 14853, USA.** *Closing date: 15th September, 1990.*

Cornell University — An Affirmative Action/Equal Opportunity Employer.

(NW5551)A

NMR —
Spectroscopist

A postdoctoral fellow position is available for motivated individual with NMR experience to join a group studying the energetics of isolated hearts and endothelial cells in culture. The newly established NMR-laboratory is well equipped (wide bore Bruker AMX 400) and there is a broad background in cardiac metabolism and cell physiology.

The salary will be ranging from £23,000-25,000 per annum.

Please send application (CV, list of publications and names of two referees) to: **Dr. Jürgen Schrader, Inst. für Herz- und Kreislaufforschung, Universität Düsseldorf, Moorenstraße 5, 4000 Düsseldorf, W-Germany.**

(W7587)A

UNIVERSITY OF EDINBURGH
Department of Molecular Biology

Applications are invited for two positions in a research group working on the molecular biology of viral hepatitis (B and C) with particular emphasis on molecular genetic approaches to studies of the immunological specificity of mutant and chimaeric antigens. One appointment is for a Technical or Postgraduate assistant (for which the salary scales are £9,705-£14,252 pa or £11,399-£13,495 pa) and the other Postdoctoral (salary scale £11,399-£18,165).

Those interested are asked to write, with a copy of their CV and the names of referees, to Professor K Murray, Department of Molecular Biology, University of Edinburgh, King's Buildings, Mayfield Road, Edinburgh EH9 3JR, from whom further information may be obtained.

Please quote reference no. 5793.

(4230)A

ENDOWED PROFESSORSHIP

The Department of Molecular Biology at Princeton University invites nominations and applications for an Endowed Professorship. Candidates should head an active research program and have an established reputation for excellence in scholarly research. Preference will be given to candiates with a strong background and research interest in biochemistry. Fo
further information, contact:

Professor Thomas Shenk
Lewis Thomas Laboratory
Department of Molecular Biology
Princeton University
Princeton, New Jersey 08544

Princeton University is an Equal Opportunity/Affirmativ
Action Employer.

(NW5565)A

UNIVERSITY OF DURHAM
Department of Chemistry
RESEARCH ASSISTANT

Required for a period of three years commencing 1 October 1990 (or
soon as possible thereafter). The project, funded by the Wellcome Tru
involves investigation of reactions of nitric oxide with haems and wi
thiols and related reactions with reference to the mechanism of vasodil
tion. Applicants should be graduates in Chemistry (or Medicinal Che
istry) and appropriate experience in the chemistry of biological syste
would be an advantage.

Salary will be £11,399 pa and it will be possible to register for a P
degree at the University.

Applications should be addressed to Dr D L H Williams, Chemistry Depa
ment, University Science Laboratories, South Road, Durham DH1 3LE, fr
whom further particulars may be obtained (tel: (091) 374 3125).

Closing date: 17th August, 1990.

(4287)A

THE UNIVERSITY OF CALIFORNIA, SAN DIEG
is seeking a
Ph.D. Applicant for a Postgraduate Researcher (PGR

Candidates should have experience in molecular biology and retr
virology or herpesvirology to conduct research in AIDS pathoge
esis and the role of viral cofactors. Position available immediate
for at least 2 years. Salary range based on University of Califor
policy. Interested individuals should send curriculum vitae with
list of three references no later than December 31st, 1990 t
Stephen A. Spector, M.D., Professor, Department of Paedi
trics, UCSD Medical Center, 225 Dickinson Street (H-814-
San Diego, CA 92103. Equal Opportunity/Affirmative Acti
Employer.

(NW5556)A

YALE UNIVERSITY SCHOOL OF MEDICINE
POSTDOCTORAL/ RESEARCH ASSOCIATE

position available immediately to study the recessive genetic mechanisms of resistance of human carcinoma cells to the antiproliferative effects of TGFβ. This question will be approached using a combination of molecular genetic techniques such as somatic cell hybridization and cDNA cloning of differentially expressed genes. Molecular biology training/experience required.

Send curriculum vitae, description of research interests, long-term goals, relevant reprints and three letters of recommendation to: **Michael Reiss, MD, Assistant Professor of Medicine, Section of Medical Oncology, Department of Medicine, Yale University School of Medicine, 333 Cedar Street, New Haven, CT 06510 (Fax #203-785-7217).**

(NW5569)A

HIV TRAINING OPPORTUNITY

Immediate openings for MD or
postdoctoral fellows interest
pathogenesis and immunolog
HIV or HIV-associated opportu
ic infections. This research
gram links the infectious dis
unit at New England Me
Center with the basic sci
retrovirology and immun
programs at Tufts Medical Sc
and is supported by a newly f
ed NIH Training Grant. If inter
please contact **Dr. Gerald Ke**
Program Director, at **617-956**
or **New England Medical C**
750 Washington Street, Bo
Boston, MA 02111. *The m*
center is an equal oppo
employer.

(NW551

M-Scan Ltd

M-Scan Ltd was founded in 1979 to provide contract biopolymer analytical services in the Biotechnology and Environmental areas. We are world leaders in the application of modern mass spectrometric methods for the analysis of protein and carbohydrate structural problems. Current instrumentation includes VG Analytical ZAB HF and ZAB 2SE mass spectrometers for high field FAB-MS, and a new electrospray instrument.

s part of the Company's continued expansion, we are seeking to fill the following posts:

PhD BIOCHEMIST

senior position requiring expertise in protein sequencing, protein chemistry carbohydrate chemistry together with mass spectrometry experience. The candidate ould possess the ability to work on his or her own initiative and interact with current nd future international clients.

GRADUATE BIOCHEMIST

his post would suit a chemist/biochemist with an interest in biopolymer structure nalysis. An enthusiastic candidate willing to learn the skills involved in protein icro-chemistry and mass spectrometry is required.

hese positions involve working both individually and within a team to solve a wide ariety of biopolymer structural problems using classical chemical methods together vith mass spectrometry.

M-Scan is situated in modern laboratories in parkland surroundings close to Ascot. We ffer attractive salaries and benefits packages based on age and experience.

pplications, including a c.v., to Dr Fiona Greer, M-Scan Ltd., Silwood ark, Sunninghill, Ascot, Berkshire SL5 7PZ. Tel: 0990 27612; Fax: 990 872709. Closing date: 24th August, 1990.

(4280)A

THE UNIVERSITY OF AUCKLAND
New Zealand

Applications are invited for the following positions:

LECTURER IN BIOLOGICAL ANTHROPOLOGY
Department of Anthropology

Biological Anthropology is taught within the Archaeology and Biological Anthropology section of the Department of Anthropology. Core papers in Biological Anthropology include Introduction to Archaeology and Biological Anthropology at Stage I, Human Evolution at Stage II, Advanced Biological Anthropology at Stage III and MA levels. Applicants should have a PhD or equivalent qualification and have teaching and research experience relevant to a university situation. Applicants should be competent in the teaching of the evolutionary biology of our species. They should also be informed of recent developments in such fields as molecular and population genetics, human palaeontology, phylogenetic patterns, cladistics, primate behaviour, human microevolution, demography, health and nutrition, and be able to apply these to teaching and research in the Pacific region. The successful applicant will be required to teach at undergraduate and graduate levels and pursue and supervise research in appropriate fields of specialisation in New Zealand and in the Pacific region.

Closing date: 17th September 1990.

LECTURER IN MOLECULAR GENETICS
Department of Cellular & Molecular Biology

Provision exists for an exceptionally well qualified candidate to be appointed at Senior Lecturer level. The Department of Cellular and Molecular Biology is well equipped for research in Molecular Biology and currently accommodates approximately 75 staff, research fellows, graduate students and technicians. In 1991 it is proposed to form a School of Biological Sciences which will incorporate the Departments of Cellular and Molecular Biology, Botany, Biochemistry and Zoology. Applicants should have a PhD or equivalent qualification and some years of postdoctoral experience. The position involves teaching and research in eukaryotic molecular genetics. We seek an appointee able to initiate a strong research programme in human molecular genetics. Opportunity will exist for the development of strong interactions with staff in the Medical School and with DNA Diagnostics Ltd, a University-based company involved in genetic testing. The successful applicant will contribute to the undergraduate and graduate teaching programme in molecular genetics and will supervise and carry out research.

Closing date: 13th September 1990.

Commencing salary will be established within the following ranges: Senior Lecturer NZ$50,000-NZ$58,600 per annum; Lecturer NZ$36,000-NZ$47,200 per annum.

Conditions of Appointment and Method of Application are available from the Appointments Officer, Association of Commonwealth Universities, 36 Gordon Square, London WC1H 0PF, UK; or from the Assistant Registrar (Academic Appointments), University of Auckland, Private Bag, Auckland, New Zealand.

The University of Auckland — An Equal Employment Opportunity Employer.

(W7591)A

SENIOR RESEARCH SCIENTIST/ PRINCIPAL RESEARCH SCIENTIST

$A41,067-$A55,230

DIVISION OF APPLIED PHYSICS
LINDFIELD, NSW, AUSTRALIA

THE FIELD: Physics of Surfaces and Thin Films.

THE POSITION: The CSIRO Division of Applied Physics supports research in a number of areas that include elements of surface science such as sputtering or evaporation of thin-film coatings for wear-resistance or optical modification, surface modification by ion bombardment, the production of thin-film superconducting devices, fine grinding and polishing of surfaces, measurement of ultramicrohardness using purpose-developed instrumentation (UMIS-2000), measurement of surface topography. The Division is now establishing a new Research Project in surface science aimed at obtaining insights into the physical properties and processes associated with thin films and the surface region of materials. It is seeking to employ an experienced scientist to explore the basic physics and materials issues that control one or more of the above research topics. This will entail interaction with other members of the Division involved in thin-films and surface science research.

THE PERSON: The successful applicant will be a person capable of leading the surface science project team. He/she will have a PhD or equivalent qualification in physics, materials science, or engineering and expertise in the mechanical properties of surfaces, including topics like: Ultramicrohardness, surface ductility, thin-film adhesion and residual surface stress, surface topography, sub-surface damage.

THE CONDITIONS: Appointment would be on an indefinite basis with Australian Government Superannuation benefits applicable.

MORE INFORMATION: A selection criteria can be obtained by telephoning Mrs Gillian Van der Eyk on (02) 413 7452 and further details of the position can be obtained by telephoning Dr Ken Hews-Taylor on (02) 413 7388.

APPLICATIONS: Applications should be framed against the selection criteria and contain relevant personal details, including qualifications and experience. The application, quoting reference No. AS90/19 and giving names of two referees, should be addressed to:

The Chief,
CSIRO Division of Applied Physics,
PO Box 218, Lindfield, NSW 2070 AUSTRALIA.

Closing date for applications: 31 August, 1990.

CSIRO
AUSTRALIA

EQUAL EMPLOYMENT OPPORTUNITY AND A NON-SMOKING WORK ENVIRONMENT ARE CSIRO POLICY.

(W7585)A

THE UNIVERSITY OF BIRMINGHAM
Department of Anatomy
TWO POST-DOCTORAL POSITIONS
REGULATORY MECHANISMS IN T-LYMPHOCYTE DEVELOPMENT

These positions are on an MRC supported 5 year programme grant to Dr E J Jenkinson, Dr G T Williams and Professor J J T Owen FRS to study regulatory mechanisms in T-cell development.

1. Will be concerned with applying molecular biological techniques to the analysis of thymocyte differentiation and selection, as an expansion of our work in this area (Nature (1986) *324*, 62. Nature (1989) *337*, 181). Experience with recombinant DNA would be an advantage, but training provided. Ref: M12100.

2. Is to study interactions between developing thymocytes and the thymic microenvironment in the control of differentiation, antigen receptor gene rearrangement and antigen receptor repertoire selection. Interest in T-cell biology, cell signalling and/or lymphokines together with practical experience in cell culture an advantage, although training provided. Ref: M12101.

Salary in the range of £10,458-£16,665 (pay award pending).

Informal enquires to 021 414 6814.

Application forms (returnable by 15th August, 1990) and further particulars available from The Director of Staffing Services, The University of Birmingham, Edgbaston, Birmingham B15 2TT. Tel: 021 414 6483 (24 hours). Quote appropriate reference number.

The University is an equal opportunities employer. (4263)A

THE INTERNATIONAL CENTRE OF INSECT PHYSIOLOGY AND ECOLOGY
PO BOX 30772, NAIROBI, KENYA

POSITIONS ANNOUNCEMENT

Applications are invited from suitably qualified candidates for the following positions tenable at Mbita Point Field Station in the CROP PESTS RESEARCH PROGRAMME.

SCIENTIST-IN-RESIDENCE (Banana Project)
(REF: SCI/90/14)

Candidates must have a PhD degree in agricultural entomology and at least three years of postdoctoral experience, as evidenced by refereed publications in the areas of ecology and control of insect pests of crops. Preference will be given to a candidate having experience in the banana weevil/nematode control.

The selected candidate will be expected to work in the area of distribution, incidence, biology, ecology and control of banana weevils including trapping for population monitoring and suppression. In addition, the selected candidate will coordinate the on-going research and training activities on the banana weevil/nematode complex in Kenya and Tanzania. The research will be carried out in collaboration with a team of scientists and graduate scholars.

SCIENTIST-IN-RESIDENCE (IPM specialist)
(REF: SCI/90/15)

Candidates must have a PhD degree in agricultural entomology followed by three years of postdoctoral experience of integrated pest management of crops. Preference will be given to candidates having had experience in the tropics.

The selected candidate will be expected to work on the development and testing of sorghum-based or maize-based IPM systems or such other systems. He will also be responsible for research and development on the integration of IPM components into crop pest control packages for use in Africa. In addition, the selected candidate will liaise with other CPRP scientific staff, other units of ICIPE, Kenya Agricultural Research Institute (KARI), Ministry of Agriculture, Government of Kenya and other regional staff as appropriate.

The remuneration package attached to the above-mentioned positions will be commensurate with the qualifications and experience of each appointee.

Applications giving detailed information on education, professional qualifications, experience, marital status, age, present terms of employment (including salary), reprints of key publications, names and addresses of four referees and photocopies of relevant certificates (which will not be returned), should be addressed to the undersigned. Please quote advert reference no. on the envelope.

The Manager for Administration and Information
The International Centre of Insect Physiology and Ecology (ICIPE)
PO Box 30772
NAIROBI, Kenya

Closing date: 31st August, 1990. (W7595)A

Leicester University

Department of Biochemistry
LECTURESHIP

This post has been made available by the appointment of Professor G C K Roberts to the Directorship of the IRC in Mechanisms in Human Toxicity and will be tenable for five years, subject to a two year probationary period.

The successful candidate will be required to teach aspects of biochemistry to undergraduate students in the Biological Sciences and Biological Chemistry degree courses, and will be encouraged to pursue an active research programme. Candidates with expertise in physical biochemistry are invited to apply, and those with experience in the biological applications of nmr spectroscopy will be especially welcome.

Initial salary, dependent on the qualifications and experience of the Lecturer appointed, will be within Lecturer Grade A (£12,086-£16,755 pa) and the post is available from 1st October 1990.

Further particulars and application forms from the **Staffing Office (Academic Appointments), University of Leicester, University Road, Leicester LE1 7RH. Tel: (0533) 522439.**

Closing date for applications: 22nd August, 1990.

(4266)A

SYSTEMS ANALYST
University of Cambridge
Salary to £20k

Post available now. Salary scale £17,500-£20,300 pa, responsible for:

1. Day-to-day administration of a Unix based network including Convex mini-supercomputer, SUN workstations and Macintoshes. Required, a strong knowledge of UNIX, FORTRAN and C. Familiarity with workstations, imaging and scientific computing networking of systems OSI and TCP/IP an advantage.
2. Developing interactive programmes for teaching and research in Geological Sciences, and providing advice and support to users of micro computers.

4 copies of applications, including cv and names of 2 referees, to the **Administrator, Department of Earth Sciences, Downing Street, Cambridge CB2 3EQ,** *by 30th September, 1990.* (4289)A

SAINSBURY LABORATORY
John Innes Centre for Plant Science Research

Applications are invited for a

TECHNICIAN

to join a team who are involved in research on plant responses to disease. Candidates should have an HNC or HND with relevant experience in a biological subject or chemistry. Salary will be on the University Technical Grade D scale: £8,904-£10,632 subject to pay award. Applications quoting reference no. SL/498 with CV and names of two referees should be submitted by 16th August, 1990, to the Personnel Officer, John Innes Institute, Colney Lane, Norwich NR4 7UH. Equal Opportunities Employer. Contributory superannuation.

(4288)A

OVER HALF
A MILLION
SCIENTISTS
ARE LOOKING
AT THIS SPACE
nature
SHOULDN'T YOU
BE FILLING IT?

UNIVERSITY OF MANCHESTER

SCHOOL OF BIOLOGICAL SCIENCES

Plant Metabolism Research Unit

2 RESEARCH ASSOCIATES

Applications are invited for the above positions, funded by SERC, each of which is tenable for up to 3 years. Project (i) "Flux control analysis of primary nitrogen assimilation in chloroplasts" will investigate the contribution that each metabolic step makes to the overall control of the conversion of nitrite to glutamate in isolated chloroplasts and intact leaf cells. Project (ii) "Pyridine nucleotide reductase and ferredoxin synthesis and control during nitrate assimilation in plant roots" will investigate the function and regulation of these proteins whose synthesis in roots is enhanced during the induction of nitrate assimilation. A good background in biochemistry/plant biology is required in each case and both projects will involve studies with purified organelles and enzymes. In the case of (ii) familiarity with molecular bilology would also be an advantage. The posts will be held in the Plant Metabolism Research Unit which comprises plant molecular biologists, biochemists, physiologists and environmental biologists in an active interdisciplinary group.

Applicants for each post should have, or expect to receive in the near future, a Ph.D. Salary in the range £11,088 to £12,381 p.a. (under review). The starting date for project (i) is 1st October, 1990 and for project (ii) 1st January, 1991 or as soon as possible thereafter in each case.

For either post a full Curriculum Vitae together with the names of two referees should be sent to Dr. M.J. Emes, Plant Metabolism Research Unit, Williamson Building, Department of Cell and Structural Biology, School of Biological Sciences, Manchester, M13 9PL.

THE UNIVERSITY IS AN EQUAL OPPORTUNITY EMPLOYER

(4278)A

STUDENTSHIPS

UNIVERSITY OF KEELE

CASE STUDENTSHIP: CANCER RESEARCH CAMPAIGN'S POLYMER CONTROLLED DRUG DELIVERY GROUP/THE WELLCOME FOUNDATION LTD

An SERC CASE studentship is available to work with Dr. Ruth Duncan (Keele) in collaboration with Tim Stanley (Wellcome) tenable from October, 1990. The project will investigate those properties of polymeric drug delivery systems that enable targeting to, and pinocytic uptake, by the lung. The successful candidate will enjoy working in an active multidisciplinary research group with many international research collaborations. Training will be provided in a number of different disciplines relevant to a career in oncology or the pharmaceutical industry and experimental techniques will include cell culture, HPLC and use of monoclonal antibodies. The basic stipend will be supplemented by an additional £1,000 and there is a generous allowance for laboratory and other expenses.

Further information from R. Duncan, tel: 0782 712810 and application forms from Dept. Academic Affairs, University of Keele, Keele, Staffs ST5 5BG.

(4246)F

The University of Sheffield

Applications are invited for a

PhD STUDENTSHIP

in the field of Oral Microbiology.

The project concerns interactions between salivary constituents and oral streptococci, with particular reference to α-amylase binding.

Applicants should hold a 1st or upper 2nd class degree, preferably in microbiology or biochemistry.

Applications including a full CV and the names of two referees should be submitted to Dr C W I Douglas, Department of Oral Pathology, Sheffield University, 31 Claremont Crescent, Sheffield S10 2TA. Tel: 0742 768555 ext 6191. Ref: R.1039/G.

(4273)F

UNIVERSITY OF CAMBRIDGE

Department of Medicine

MRC RESEARCH STUDENTSHIP

An MRC Studentship, awarded under the AIDS Directed Programme, is available (due to the unavoidable withdrawal of a previous candidate) to work with Professor J G P Sissons and colleagues in the area of viral immunity and pathogenesis.

Candidates should possess or expect to gain at least a 2:1 or equivalent degree.

Interested applicants should telephone Professor J G P Sissons, Department of Medicine, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ (telephone Cambridge (0223) 336849) for further details as soon as possible.

The University follows an equal opportunities policy. (4253)F

FELLOWSHIPS

LEUKAEMIA RESEARCH FUND CENTRE at the INSTITUTE OF CANCER RESEARCH

POST-DOCTORAL RESEARCH FELLOW

Fulham Road, London SW3

Applications are invited for a post-doctoral fellowship to investigate the expression of cell adhesion molecules by haemopoietic stem cells and their relevance to haemopoietic differentiation, bone marrow transplantation and leukaemia.

Applicants should have, or expect to obtain shortly, a PhD in a biological subject. Experience with cell biological, biochemical and/or tissue culture techniques would be an advantage.

Salary will be in the range of £12,108 to £17,022, according to experience. The post is for a three year period in the first instance.

To apply send a curriculum vitae, in duplicate, with the names and addresses of two referees to the Personnel Officer, Institute of Cancer Research, 17A Onslow Gardens, London SW7 3AL quoting reference number 7.90.S.N.18.

Informal enquiries may be made to Dr M Y Gordon, Leukaemia Research Fund Centre (Tel: 071-352 8133).

Applicants are advised that smoking is prohibited in the majority of the Institute's premises. (4252)E

JOHN INNES CENTRE FOR PLANT SCIENCE RESEARCH

PLANT MOLECULAR PATHOLOGIST

Applications are invited for a three year post doctoral fellowship to carry out research into the molecular pathology of maize streak virus. Applicants should have experience in molecular biology and a knowledge of plant tissue culture would be advantageous. The post is supported for three years by the Rockefeller Foundation and the appointment will be made at an appropriate point on the University R & A Scale II with a salary in the range £15,372 to £20,469 (pay award pending). Contributory Superannuation Scheme. Equal Opportunities Employer.

Further information can be obtained from Professor J W Davies.

Applications quoting reference no VIR/501 should include a curriculum vitae and the names of two academic referees and be sent to the Personnel Officer, John Innes Institute, John Innes Centre for Plant Science Research, Colney Lane, Norwich NR4 7UH.

Closing date: 24th August, 1990.

(4271)E

UNIVERSITY OF EDINBURGH

Department of Preclinical Veterinary Sciences

POSTDOCTORAL RESEARCH FELLOWSHIP IN NEUROSCIENCE

A Postdoctoral Research fellow is required to join a MRC Programme Research Grant team studying the anatomical and functional properties of identified neurons in the spinal cord. Experience of in vivo neurophysiological techniques is required, preferably including intracellular as well as extracellular microelectrode methods and ionophoresis.

The post will be available from 1st October 1990 and is funded to 31st December 1993 on the grade 1A Scale (£11,399-£18,165).

Further information may be obtained from, and applications (including a CV and the names of two referees) should be sent to: **Professor A G Brown and Dr D J Maxwell, Department of Preclinical Veterinary Sciences, University of Edinburgh, Summerhall, Edinburgh EH9 1QH.**

Please quote reference no. 5789.

(4174)E

Post Doctoral Research Fellowships

ICI Pharmaceuticals is one of ICI's major international business areas... with an outstanding record of research in and development of, novel medicines. Our commitment to scientific excellence and innovative research has already resulted in market leading products which include anti-infectives, anaesthetics, anti-cancer agents and cardiovascular drugs. As part of our continued commitment to high level research, we are now offering two 2 year Post Doctoral Research Fellowships in our Bioscience Department. These are excellent opportunities for young PhD scientists to work on important and well-funded research programmes in well-equipped modern laboratories at our research complex in rural Cheshire.

The role of Phosphatidylinositol-3-kinase in cell growth

Phosphatidylinositol-3-kinase (PI-3-kinase) is activated by platelet derived growth factor receptor and appears to play an important role in signal transduction leading to DNA synthesis in PDGF treated fibroblasts. Mutation studies of the PDGF receptor have distinguished a predominant role for PI-3-kinase in this process in contrast with PI-4-kinase, the enzyme which has classically been associated with initiation of inositol phospholipid signalling mechanisms.

You will be expected to investigate the significance of PI-3-kinase-mediated signalling in the control of tumour cell proliferation. Individuals with experience in enzymology and/or the biochemical analysis of signal transduction pathways are particularly encouraged to apply.

The appointment will be for 2 years. An attractive salary will be offered, commensurate with experience. Some assistance will be available for relocation to this attractive part of Cheshire.

Interested candidates should write with full c.v. quoting reference AW/AS to: The Personnel Department, ICI Pharmaceuticals, Mereside, Alderley Park, Nr. Macclesfield, Cheshire SK10 4TG.

(4270)A

ICI Pharmaceuticals

Scottish Marine Biological Association

MOBIL FELLOWSHIP

Applications are invited fo
postdoctoral Fellowship
the effects of stress on ec
noderms. Of particular int
est are the effects of h
stress on the symbiotic s
cuticular bacteria. This int
disciplinary project will p
ticularly suit a candidate w
experience in microbiolo
biochemistry or immun
logy, though candidates fr
other areas may apply.

The Fellowship is funded
Mobil North Sea Ltd for thr
years and forms part of t
SMBA research programn
based at the Dunstaffna
Marine Laboratory. Sala
will be in the range of £10,6
to £14,909 per annum.

**Further details of the Labo
tory, the project and con
tions of service may be o
tained from the Direct
SMBA, PO Box 3, Ob
Argyll, Scotland PA34 4AD**

*Closing date for applicatio
is 17th August, 1990.* (4259)E

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THE OPEN UNIVERSITY
FACULTY OF SCIENCE
Department of Earth Sciences
POST DOCTORAL RESEARCH FELLOWSHIPS IN EARTH SCIENCES

Applications are invited for one, and possibly two, two year post doctoral research fellowships in Earth Sciences from 1st October, 1990. These fellowships will enable young scientists to undertake innovative research projects at the Open University. A brief outline of the proposed research should be submitted with the application form. Candidates are invited to discuss possible projects with members of Earth Sciences staff at the University if that would be helpful — telephone (0908) 652889.

Candidates will be required to have completed a PhD before the appointment date. The initial salary will be in the range £10,458-£16,565 (under review) of the 1A scale for research and analogous staff. It is expected that appointment(s) will be made at the lower end of the scale.

Application forms and further particulars are available from Ann Hall (Ref: 8050/2), Faculty of Science, The Open University, Walton Hall, Milton Keynes MK7 6AA, or telephone (0908) 655938. There is a 24 hour answering service on (0908) 653868.

The closing date for the receipt of applications is 1st September, 1990.

Equal Opportunity is University Policy. (4272)E

CHRIST'S COLLEGE, CAMBRIDGE
AMERSHAM SENIOR RESEARCH FELLOWSHIP IN BIOCHEMISTRY

Applications are invited for a Senior Research Fellowship in Biochemistry at Christ's College, founded by Amersham International plc.

The Fellowship is intended for a biochemist of exceptional ability who is well qualified for an academic, industrial, or research career, but who does not hold a tenured post at the time of application; it is also suitable for someone on leave from a tenured post who wishes to concentrate on research for a period. It is hoped that the successful candidate, who would normally not be aged above 40, would take up the Fellowship during 1991, and would hold it for a maximum of four years. Although the choice of a research topic would be left to the candidate, preference will be given to candidates who wish to carry out research involving radioactively labelled compounds, or applications technology. Candidates will normally be expected to work within the University Department of Biochemistry.

The stipend offered is incremental within the range of the University Lecturers' scale (£11,088 × 6 — £14,703, currently under review), the starting point depending on age and experience; for an unmarried Fellow, accommodation in College may be available, with some adjustment in stipend. The Fellow would be entitled to seven free meals per week (either lunch or dinner) and a Commons Allowance.

Forms of application may be obtained from Professor Sir Hans Kornberg, FRS, The Master, Christ's College, Cambridge CB2 3BU, to whom completed applications should be returned by Friday, 28th September, 1990. (4254)E

EMBO

European Molecular Biology Organization

LONG TERM FELLOWSHIPS IN MOLECULAR BIOLOGY

AUTUMN 1990 AWARDS

Next deadline: 15 August 1990

EMBO long term post-doctoral fellowships are awarded to promote the development of molecular biology and allied research in Europe and Israel. To be eligible a candidate must hold a doctorate degree and the exchange must involve a laboratory in Western Europe or Israel. EMBO fellowships are not, however, awarded for exchanges between laboratories within any one country. Long term fellowships are usually awarded initially for one year, but with the possibility of renewal for a second year. In cases of exceptional scientific merit, however, the selection committee can award a 2-year fellowship. The fellowship comprises a return travel allowance for the fellow and any dependents and a stipend and dependents' allowance.

Since the selection procedure may include an interview, candidates are requested to respect the deadline for complete applications which is 15 August 1990. Successful candidates will be notified of their awards immediately after the meeting of the selection committee, which is on 29 October 1990.

Application forms and further details may be obtained from **Dr. J. Tooze, Executive Secretary, European Molecular Biology Organization, Postfach 1022.40,69 Heidelberg 1, F.R. Germany.** Inquiries should be accompanied by a self-addressed adhesive label. (W7235)E

THE PATRICK GROVE MEMORIAL FELLOWSHIP

For research into clinical diagnostic and therapeutic work with radionuclides.

Amersham International have funded this Fellowship to encourage young men and women to pursue original work in the United Kingdom. Applications are invited from heads of departments and others in appropriate administrative positions in the United Kingdom, or from individuals world wide (who should enclose a supporting letter from the United Kingdom department in which they wish to work).

The application should include a full curriculum vitae of the candidate, who should be a medical or non-medical graduate. It should state the purpose, background and plan of investigation of the projected research, and explain how this would fit into existing and projected work in the parent department.

This Fellowship is for three years and will cover salary and "on costs" in the region of £20,000 pa together with modest departmental expenses.

Closing date: 1st September, 1990.

Reply to: **Professor GH du Boulay, CBE, The Radiological Research Trust, 36 Portland Place, London W1N 3DG.** (4245)E

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Departments of Biochemistry and Genetics

RESEARCH FELLOWSHIP IN TRANSGENIC ANIMAL TECHNOLOGY

Applications are invited for a five-year Research Fellowship in transgenic animal technology. This is a joint appointment between the Departments of Biochemistry and Genetics, funded by earmarked industrial sponsorship. The two closely aligned Departments have strong research records and a common interest in utilising and developing transgenic techniques to study mammalian gene-expression and function.

Post-doctoral candidates with experience of molecular biology and transgenic animal techniques will be welcomed. The necessary equipment, facilities and basic running costs are available. The successful candidate will be encouraged to develop his/her own research projects, but there are opportunities for interaction with other groups interested in gene-manipulation in ES cells and the construction of transgenic models of human diseases.

The salary will be on the 1A scale, £11,399 to £18,165, and the appointment is available from 1st October, 1990.

Applications, including a curriculum vitae of two academic referees, should be sent to **Professor W J Brammar, Department of Biochemistry, University of Leicester, University Road, Leicester LE1 7RH, by 23rd August, 1990.** (4290)E

SKIN CANCER RESEARCH

POST DOCTORAL FELLOWSHIP

University of Alberta

Two positions are available immediately to study molecular biology and immunobiology of UV-photocarcinogenesis and TIL (tumor infiltrating lymphocytes) of skin cancer. No previous experience in skin cancer research required. The amount awarded will be equivalent to MRC and NCI guidelines, with future opportunities to apply for independent research and faculty positions.

Send a resume and three references to: **Kowichi Jimbow, MD, PhD, Professor and Director, Division of Dermatology and Cutaneous Sciences, 260G Clinical Research Wing, Heritage Medical Research Center, University of Alberta, Edmonton, Alberta T6G 2S2.**

The University of Alberta is committed to the principle of equity in employment.

(NW5573)E

THE INSTITUTE OF CANCER RESEARCH

POSTDOCTORAL RESEARCH FELLOWSHIP

Sutton, Surrey

A Postdoctoral Research Fellowship within the Academic Department of Haematology and Cytogenetics (Professor D Catovsky) is available immediately for an initial period of two years for work on the molecular cloning and expression of genes involved in the pathogenesis of human lymphoid malignancies.

Candidates should possess a PhD in molecular biology and be familiar with current techniques for gene cloning and DNA analysis. The successful candidate will join a multi-disciplinary team based at Sutton, Surrey. The post is supported by a grant from the Kay Kendall Leukaemia Fund.

Salary will be in the range of £14,031 pa to £17,022 pa inclusive, according to experience. (Pay award pending).

Applications, with full curriculum vitae and addresses of three referees, should be sent to The Personnel Officer, Institute of Cancer Research, 17A Onslow Gardens, London SW7 3AL, quoting reference numbers 8.90.S.N.20. (4291)E

MAFF Ministry of Agriculture Fisheries and Food

OPEN CONTRACTING SCHEME 1991/92

The Ministry of Agriculture, Fisheries and Food intends to commission research projects in the areas listed below.

Proposals are invited for short term R&D projects (1–3 years) on the following topics to begin in 1991/92.

A Studies on recent and emerging animal disease conditions including campylobacteriosis and BSE.

B Evaluation of the welfare of pigs in different growing/fattening systems.

C Evaluation of mortality and injury to laying hens in commercial alternatives to the battery system.

D Investigation of welfare problems in turkeys associated with stocking densities, leg weakness and vices.

E Manipulation of nutritional and environmental factors in broiler production in order to reduce live bird and carcass contamination.

F Engineering studies on silage effluent containment and its disposal.

G Levels, availability to food chain and impact on soil microflora or organic contaminants in different types of soil, derived from sewage sludge.

H Loss of nitrate during water movement between agricultural soil and unconfined aquifer boreholes.

I Modification of rapeseed or rapeseed oils to produce oils with wider applications.

J The biochemical, microbiological or physical properties of peat alternatives: studies aimed at improving their consistency and reliability as a horticultural growing medium.

K Processes influencing the stability of coastal saltings in the context of coast defence.

L Microbiology of farm wastes with respect to water quality protection.

M Climate Change:
  (i) Climate sensitivity of UK farming systems.
  (ii) Implications of likely changes in climatic extremes for agriculture.
  (iii) Impact on soil water and thermal regimes, and nitrogen balance.
  (iv) Impact of CO_2 and water regime changes.
  (v) Insect pests and disease vectors.
  (vi) Physiological studies on methane emission from cattle.
  (vii) Improved estimation of N_2O emissions from arable and pastoral soils.

N Organic Farming:
  (i) Nutrient availability, organic matter inputs and soil condition in relation to environmental pollution and soil protection.
  (ii) The use of green manures for horticultural crops.
  (iii) Mechanical weed control techniques for agriculture and horticulture.
  (iv) Effects of organic farming on desirable and undesirable plants and animals (including insects and, particularly, birds).

Applications with an element of industrial collaboration, where relevant, are particularly welcome.

Application forms and further details on the topic areas are available from: Dr Bill Woodbridge, Ministry of Agriculture, Fisheries and Food, Room G15, Nobel House, 17 Smith Square, London SW1P 3JR (Tel: 071-238 5512).

Closing date: 15th October 1990.

(4258)G

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PRELIMINARY ANNOUNCEMENT

150th ANNIVERSARY CONFERENCE ON THE PERMIAN SYSTEM

August 1991—Perm, USSR

Sponsor: USSR Academy of Science, Uralian Group

All aspects of the Permian System will be covered

Abstracts and Proceedings of the Conference will be published in English

Circular available late September 1990:
- General information
- Dates of meeting and field excursions
- Registration and costs
- Call for papers and deadlines

Request circular from:
Prof. A. E. M. Nairn, Perm Conf.
Earth Sciences & Resources Inst.
University of South Carolina
Columbia, SC 29208 USA
Telephone: 803-777-6484
Telex: 9102501347 USC ESRI UQ
Fax: 803-777-6437

(NW5554)C

BIOTECH USA

The 7th Annual Industry Conference and Exhibition Sponsored by *Bio/Technology* and Conference Management Corp.

NOVEMBER 27–29, 1990
WASHINGTON D.C.

CALL FOR PAPERS AND POSTERS
on the following topics

Pharm/Biotech:
- Producing Pharmaceuticals in Plants and Animals
- Rational Drug Design
- Fermentors for Biopharmaceutical Production
- Protein Purification Techniques
- Diagnostics and Devices

Ag/Biotech:
- Engineering Plants for Pest and Disease Resistance
- Agronomic Improvement of Livestock
- Food Engineering

Please send abstracts by September 1 to:

Gina Amatruda
CMC/BIOTECH USA
200 Connecticut Ave.
Norwalk, CT 06856–4900

(NW5516)C

Royal Postgraduate Medical School
(University of London)

In Situ Hybridisation Course

22-24 October, 1990

A short intensive course based on a practical approach in the laboratory and on informal workshop lectures. Applicants will be accepted on a first come, first served basis and thus early application is advisable.

Topics covered will include:
- ★ *Tissue collection, fixation and sectioning.*
- ★ *Labelling of RNA and DNA probes using radiolabelled and biotinyalted nucleotides.*
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- ★ *Autoradiography.*
- ★ *Chromogenic detection systems.*
- ★ *Interpretation and specificity of results.*
- ★ *Combined* in situ *hybridisation and immunocytochemistry techniques.*
- ★ *Quantification of data.*

Course organisers: Julia M Polak, Giorgio Terenghi and Sally Gibson (Histochemistry)

Course fee (inc. catering excl. accommodation: £220.00

Further details and application forms from:
Wolfson Conference Centre, Royal Postgraduate Medical School, Hammersmith Hospital, Du Cane Road, London W12 0NN.
Telephone: 081-740 3117 **Fax:** 081-740 4950
Deadline: 20th September, 1990.

(4234)C

8th CONFERENCE ON PROTEOLYSIS
ICOP-Meeting 1990

October 14-19,
Munich/Wildbad-Kreuth near Tegernsee

Organizers: H Fritz (Munich), H Holzer (Freiburg), M Jochum (Munich), H Kirschke (Halle), D H Wolf (Stuttgart).

Topics: Proteolysis in microbial and mammalian cells; — structure, mechanism of action and gene regulation of proteinases and inhibitors; — protein catabolism: cytosolic, ER and lysosomal pathways; — limited proteolysis in organelle biogenesis and hormone activation; — proteolysis in disease.

Fee: DM 1.500,– Research associates; DM 2.000,– Industry associates.

The fee includes registration, lodging and meals.

A limited number of posters can be accepted. Applications should be sent until August 31st, 1990, to: Prof H Fritz, Dept Clinical Biochemistry, University of Munich, Nussbaumstraße 20, D-8000 München 2, FRG. (W7582)C

continued over

ADVANCES IN INFLAMMATION

The Impact of New Developments
31st October-1st November, 1990.

The Royal College of Physicians, London
By Kind Permission of the Treasurer

This major international conference will cover our current understanding of the mechanisms of inflammation, and examine in depth:

- ★ *The development of new models for our understanding of the inflammatory response.*
- ★ *How advances in immunology and moleculary biology are pointing to the emergence of novel and powerful anti-inflammatory agents.*
- ★ *The exact role of the intercellular messengers in inflammation.*

For a full conference programme/registration details of the above events, please contact:

Renata Duke, IBC Technical Services Ltd,
Bath House (3rd Floor)
56 Holborn Viaduct
London EC1A 2EX
Tel: 071-236 4080
Fax: 071-489 0849
Telex 888870 IBC G

(4283)C

AN INTERNATIONAL CONFERENCE ON THE LARGE MARINE ECOSYSTEM CONCEPT AND ITS APPLICATION TO REGIONAL MARINE RESOURCE MANAGEMENT

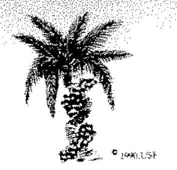
CONVENED BY: National Oceanic and Atmospheric Administration (NOAA), U.S. Dept. of Commerce; World Conservation Union (IUCN); Intergovernmental Oceanographic Commission; International Council for the Scientific Exploration of the Mediterranean (ICSEM), Monaco. **SPONSORED BY**: American Association for the Advancement of Science (AAAS); National Science Foundation (NSF); United Nations Environmental Program (UNEP); Food and Agricultural Organization of the UN; Council for Ocean Law; World Bank; Marine Mammal Commission; Scientific Committee on Oceanic Research (SCOR); International Coastal & Oceans Organization; and the Musee Oceanographique de Monaco

1-6 October 1990 - Monaco

Patron: H.S.H. The Prince Rainier III
Hosted by: The Musee Oceanographique de Monaco and the Principality of Monaco

Participation is open to biological and physical scientists, economists, lawyers, geographers, managers, and students interested in marine resources. For list of topics and registration forms, please contact: D. Elder, Coord. Mar. Programme, World Cons. Union, Avenue du Mont-Blanc, CH-1196 Gland, Switzerland; Tel: (41 22) 649114; Fax: (41 22) 642926 or L. Hedrick, NOAA, NMFS, Narragansett Lab., Narragansett, RI 02882 USA, Tel: 401-782-3211; Fax: 401-782-3201.

(NW5570)C



IUB Conference on NUCLEIC ACID THERAPEUTICS

January 13-17 1991
Clearwater Beach, Florida

DNA and RNA analogs are potential gene-specific therapeutics against cancers, viruses, and parasites. This meeting provides a forum for investigators from academia, industry, and government to share the most recent developments in Oligonucleotide Derivatives, Cellular Uptake, Animal Pharmacology, Anticancer Applications, Antiviral Applications, Commercialization and Regulation.

Participants will be limited to 300.

To apply, please send research interests, list of relevant papers, and camera-ready or ASCII abstract, to Dr Eric Wickstrom, Department of Chemistry, University of South Florida, Tampa, Florida 33620 USA, by September 30, 1990. Upon acceptance, registration and meals fee of $300 will be due by December 1, 1990.

(NW5575)C

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BERLIN
August 26th-31st, 1990

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THE AMERICAN ASSOCIATION FOR CANCER RESEARCH

announces

AACR Special Conference in Cancer Research

CHROMOSOMAL AND GROWTH FACTOR ABNORMALITIES IN LEUKEMIA

October 14-18, 1990, Chatham Bars Inn, Chatham (Cape Cod), Massachusetts

SUPPORTED BY A GENEROUS GRANT FROM THE GENERAL MOTORS CANCER RESEARCH FOUNDATION

PROGRAM COMMITTEE Janet D. Rowley, Chicago, IL; Peter C. Nowell, Philadelphia, PA; Donald Metcalf, Melbourne, Australia; Leo Sachs, Rehovot, Israel.

SCIENTIFIC PROGRAM

Sunday Evening, October 14

KEYNOTE ADDRESS George Klein, Stockholm, Sweden

Monday Morning, October 15

REGULATION OF NORMAL AND LEUKEMIC LYMPHOID CELLS William E. Paul, Bethesda, MD; Tadamitsu Kishimoto, Osaka, Japan; Irving L. Weissman, Stanford, CA; Thomas A. Waldmann, Bethesda, MD; Suzanne Cory, Melbourne, Australia.

Monday Afternoon, October 15

GENETIC ANALYSIS OF CHROMOSOMAL ABNORMALITIES IN LYMPHOID NEOPLASMS Stanley Korsmeyer, St. Louis, MO; Timothy McKeithan, Chicago, IL; Michael Cleary, Stanford, CA; Carlo M. Croce, Philadelphia, PA; Terry Rabbitts, Cambridge, England.

Tuesday Morning, October 16

MYELOID GROWTH FACTORS Leo Sachs, Rehovot, Israel; Charles J. Sherr, Memphis, TN; Nicos Nicola, Melbourne, Australia; David W. Golde, Los Angeles, CA.

Tuesday Afternoon, October 16

GROWTH FACTORS IN MYELOID LEUKEMIA Donald Metcalf, Melbourne, Australia; Constance J. Eaves, Vancouver, Canada; James D. Griffin, Boston, MA; Joseph Lotem, Rehovot, Israel.

Wednesday Morning, October 17

GROWTH FACTORS IN MYELOID LEUKEMIA (Continued) Nicholas Gough, Melbourne, Australia; James Ihle, Memphis, TN; Cila Blatt, Rehovot, Israel; Jerry Adams, Melbourne, Australia; Roland Mertelsmann, Mainz, West Germany.

Wednesday Afternoon, October 17

GENETIC ANALYSIS OF CHROMOSOME REARRANGEMENTS IN MYELOID LEUKEMIA Owen Witte, Los Angeles, CA, Gerard Grosveld, Rotterdam, Netherlands; Janet D. Rowley, Chicago, IL; Michelle LeBeau, Chicago, IL.

Thursday Morning, October 18

INTEGRATION AND SUMMARY Peter C. Nowell, Philadelphia, PA; Michael B. Sporn, Bethesda, MD; John Cairns, Boston, MA.

INFORMATION AND APPLICATION FORMS: **American Association for Cancer Research, Public Ledger Building, Suite 816, Sixth and Chestnut Streets, Philadelphia, PA 19106.** 215-440-9300 215-440-9313 (FAX).

(NW5566)C

GRANTS & SCHOLARSHIPS

AMYLIN SYMPOSIUM

La Jolla, California, January 8-9, 1991

Organizers:

Garth J.S. Cooper, Timothy J. Rink, Kenneth L. Luskey

This Symposium will cover the emerging picture of the properties and functional significance of this newly discovered peptide hormone. Invited chairpersons and speakers include the following:

Amylin & Amyloid:

Garth Cooper, Amylin Corporation
Per Westermark, University of Uppsala
Nick Hales, University of Cambridge

Molecular & Cell Biology of Amylin:

Sydney Brenner, University of Cambridge
Ken Luskey, University of Texas
Donald Steiner, University of Chicago
Garth Cooper, Amylin Corporation

Amylin & Fuel Metabolism:

Philip Randle, John Radcliffe Hospital
Andrew Young, Amylin Corporation
Dennis McGarry, University of Texas
Brendan Leighton, University of Oxford

Diabetes & Insulin Resistance:

Roger Unger, University of Texas
Jose Caro, East Carolina University
Ed Horton, Medical Center Hospital of Vermont

The Symposium will be in the Conference Center of the Embassy Suites Hotel at 4550 La Jolla Village Drive, San Diego, California 92122. A registration fee of $295 will cover: Attendance, Welcome Reception, Lunch on both days, Conference Dinner. A Poster Session is planned. Participants are invited to present their abstracts. Favorable terms for accommodations have been arranged through Embassy Suites Hotel, and meetings saver fares will be available through American Airlines. Please call the toll-free number: (800) 433-1790 at American when making reservations, and refer to Star File Number S01118C. Space is limited, so early registration is recommended.

For information on registration contact:

AMYLIN

Ms. Lisa Dimler
Amylin Corporation
9373 Towne Centre Drive, Suite 250
San Diego, California, USA 92121
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(NW5571)M

NATURE

120 YEARS AGO

An experiment, performed by M. J. M. Philipeaux, in which he transplanted one of the incisor teeth of a guinea-pig into the comb of a cock, has been referred to by M. P. Bert in his "Thesis on Animal Grafting". M. P. Bert provides the following observations:– On January 13, 1853, M. Philipeaux, after having made an incision into the head of a young cock, introduced into it the incisor tooth of a guinea-pig that had been born a few hours previously. The tooth, very complete and furnished with its bulb, was so placed that the bulb was at the bottom of the wound and the extremity of the tooth was eight millimetres long and two millimetres thick. The cock was killed ten months after the operation. The tooth, which, on the day of operation, was entirely concealed and covered in the wound, projected, when the animal was killed, five millimetres from the surface. M. Philipeaux had dissected out in the specimen the whole length of the tooth, and found that it measured no less than thirteen millimetres; it had consequently grown five millimetres. The interest of this experiment, which in other respects resembles those of Hunter and Sir A. Cooper, in which the spur of the cock was transplanted to the comb, consists in the circumstance that here a graft was accomplished in one animal of a part belonging to another, belonging to an entirely different zoological class.

The Abbé Moigno announces in the last number of *Les Mondes* that the stone knives with which Joshua performed the rite of circumcision have been found in his tomb by the Abbé Richard. The account, which concludes as follows, we commend to the special attention of Mr. John Evans and Mr. Sorby:– "Voici donc qu'un des faits historiques les plus singuliers de la Bible a reçu la confirmation la plus éclatante, et que nous entrons en possession de silex taillés il y a 3,550 ans, plus vieux bien certainement, nous le prouverons jusqu'à l'évidence, (!) que les silex taillés de la vallée de la Somme ou des grottes d'Aurignac. Qui sait même si le spectroscope manié par des mains aussi habiles que celles de M. Sorby ne nous démontrera pas la présence, sur quelques-uns de ces silex, du sang de la circoncision." (!!)
From *Nature* July 28, 1870.

100 YEARS AGO

Perhaps Mr. Stanley or Surgeon Parke, if applied to, could throw some more light on the extraordinary statement made by Emin Pasha, recently referred to, which, if it be true, is the most important statement in the whole book.

It is probable that when Emin Pasha witnessed the torch-bearers, whether chimpanzees or young negroes or dwarfs, he was not alone, and, even though very short-sighted, he would have been able to verify his observation of the torch-bearing animals by reference to those near him. An experienced naturalist like Emin Pasha is not likely to have made the mistake Professor Romanes thinks he did make — but it is possible.

Bearing in mind that a large ape is now undoubtedly acting as a signalman (under direction) on a railway at Natal, who can say what the limits of intelligence are in the tribes of Simians?
"Chimpanzees and Dwarfs in Central Africa", J. F.
From *Nature* July 24, 1890.

50 YEARS AGO

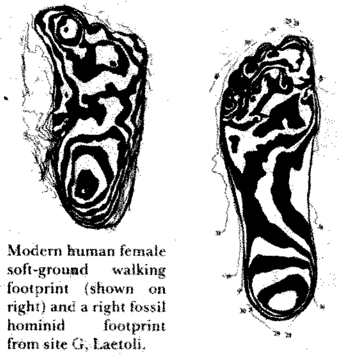
In the 'twenties I sent to the British Museum (Natural History) from this place, a distance of about 7,000 miles, two or three specimens of *Cephalorhynchus commersonii*, a dolphin 3–4 ft in length, preserved with common salt. The abdominal cavity was opened and salt stuffed in to capacity and the whole animal was covered with a heap of salt. Much of the fluid resulting
possible was drained off before packing
patch. The method proved entirely satisfa
being, I believe, the original of the cast (♂
the Museum.

Salt was used for the preservation of the
byronia illustrated in my recent paper on th
was about 14 cm long and 11 cm across
very muscular. It was placed in a glass ja
fresh salt being added to keep the specime
brine was formed which was poured off. M
specimen was taken from the salt, soaked
until quite soft, and then very gradually
about 70 per cent. It remained perfectly fl
able subject for dissection.
"Common Salt as a Preservative", J. E.
Department, Falkland Islands.

The 'Iron Library' extension, which is no
British Museum, London, by H. M. Office
ing development in the introduction of
present structure is in the nature of an in
example of an all-welded structural fram
Office of Works. The extension occupies th
the rectangular plot surrounding the hi
Room and replaces a part of the existing
and timber.
From *Nature* July 27, 1940.

10 YEARS AG

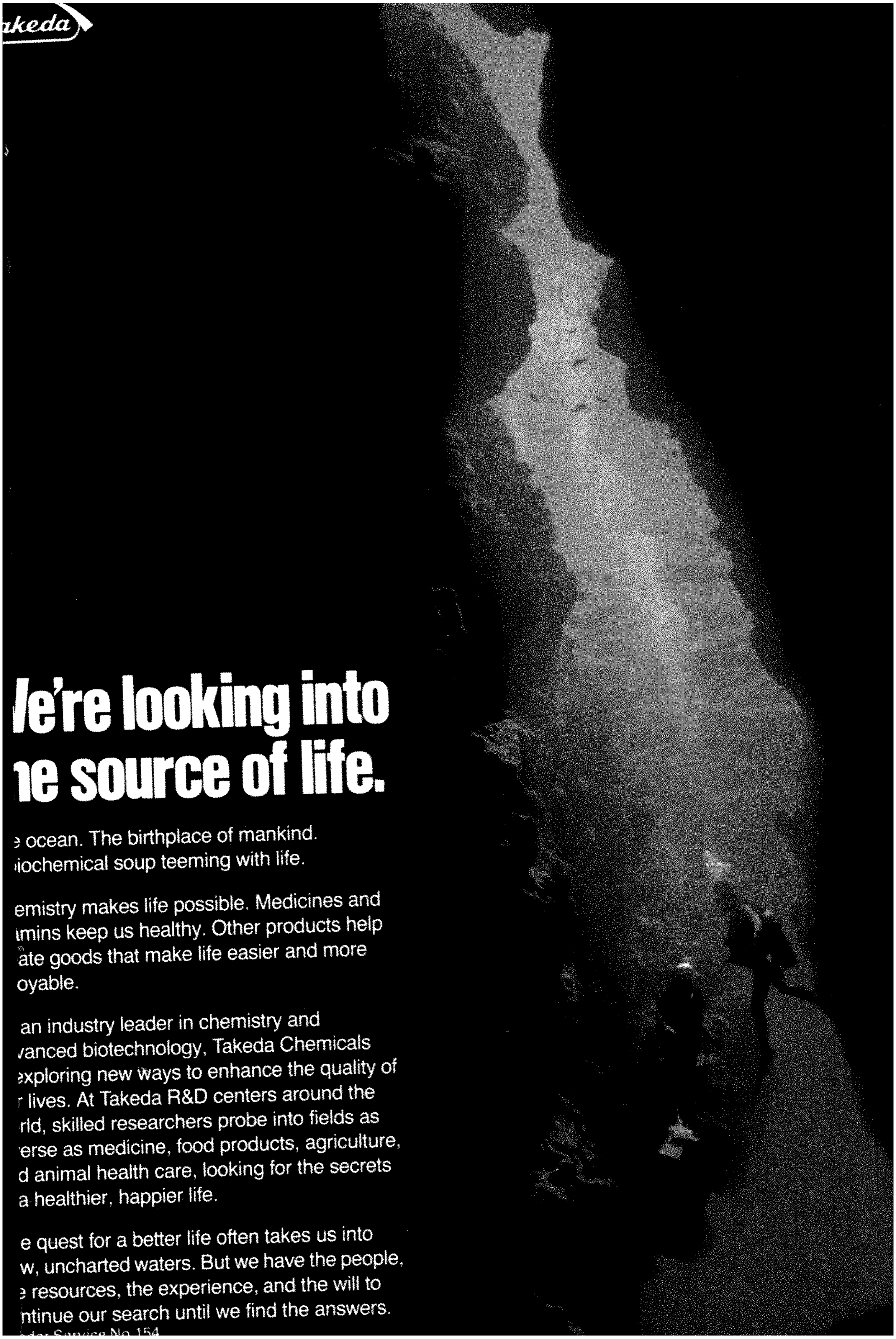
The ability to stand upright and walk on
nized as a crucial hominid adaptation that
the course of human evolution. The relief
the task of supporting the trunk in loco
mitted the efficient carriage of food, infa
weapons. Any or all of these activities c
improved the differential survival of ea
when upright stance and bipedal gait were
hominid evolutionary line is not known,
apparently bipedal footprints of Pliocene
Tanzania is important in itself. A study of
here, has
hominid
mitted t
forces
ground
to that o
The f
individu
grained
sequent
haps afte
into a tu
often in
Because
walking reflect the way in which weight a
transmitted to the ground, we have compa
with those of modern man made exper
similar consistency. Modern human footpr
ing over wet sand (grade 25/52, that is 0.3
the laboratory so that clear impressions we
stereophotography.


Modern human female soft-ground walking footprint (shown on right) and a right fossil hominid footprint from site G, Laetoli.

"Laetoli Pliocene hominid footprints and
and E. H. Wickens.
From *Nature* July 24, 1980.

IN NEXT WEEK'S

**A 1,400-year record of tree rings and
and the weak interaction ■ Adhesi
immune system ■ Large-scale structu
MHC molecules fold in the cold ■
defence.**



ıkeda

/e're looking into
ıe source of life.

ɜ ocean. The birthplace of mankind.
ıiochemical soup teeming with life.

emistry makes life possible. Medicines and
ımins keep us healthy. Other products help
ate goods that make life easier and more
oyable.

an industry leader in chemistry and
ʻanced biotechnology, Takeda Chemicals
ɜxploring new ways to enhance the quality of
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rld, skilled researchers probe into fields as
erse as medicine, food products, agriculture,
d animal health care, looking for the secrets
a healthier, happier life.

e quest for a better life often takes us into
w, uncharted waters. But we have the people,
ɜ resources, the experience, and the will to
ntinue our search until we find the answers.

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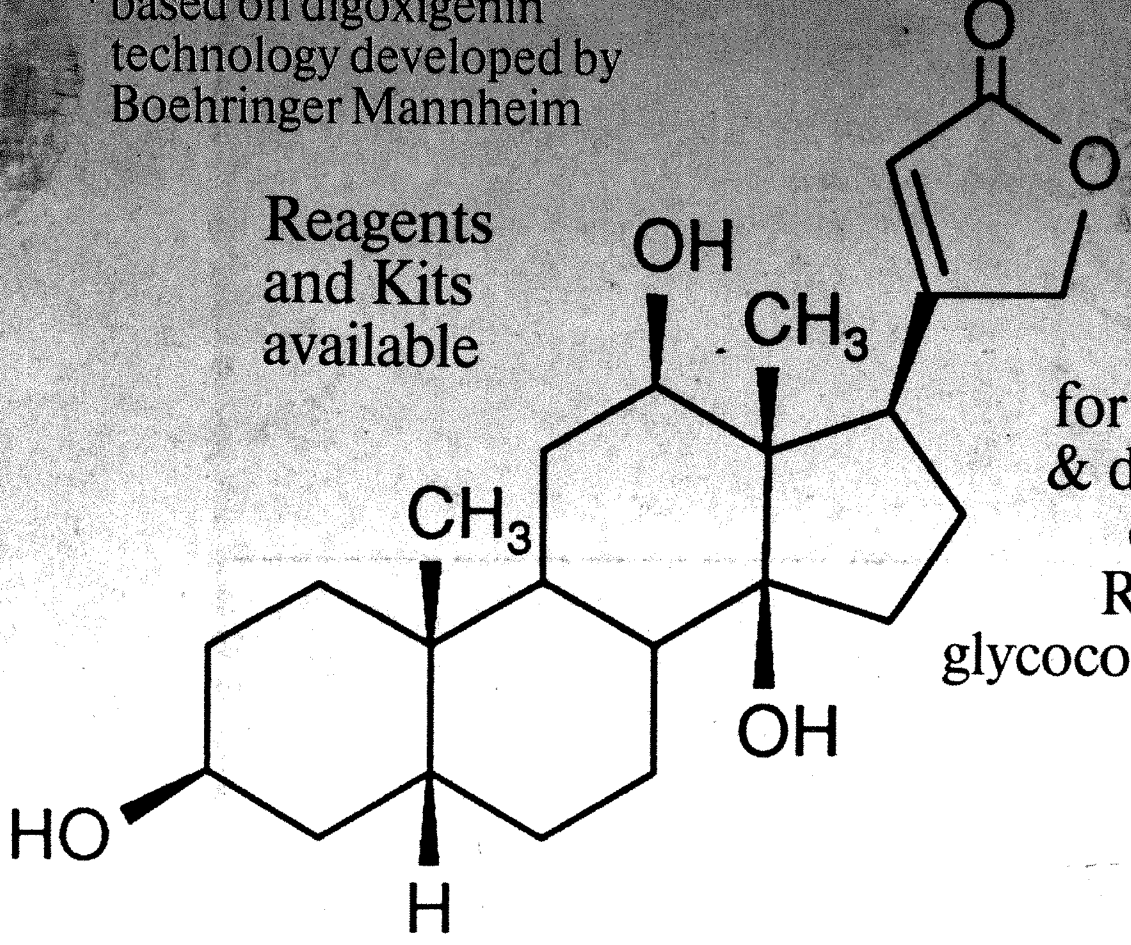

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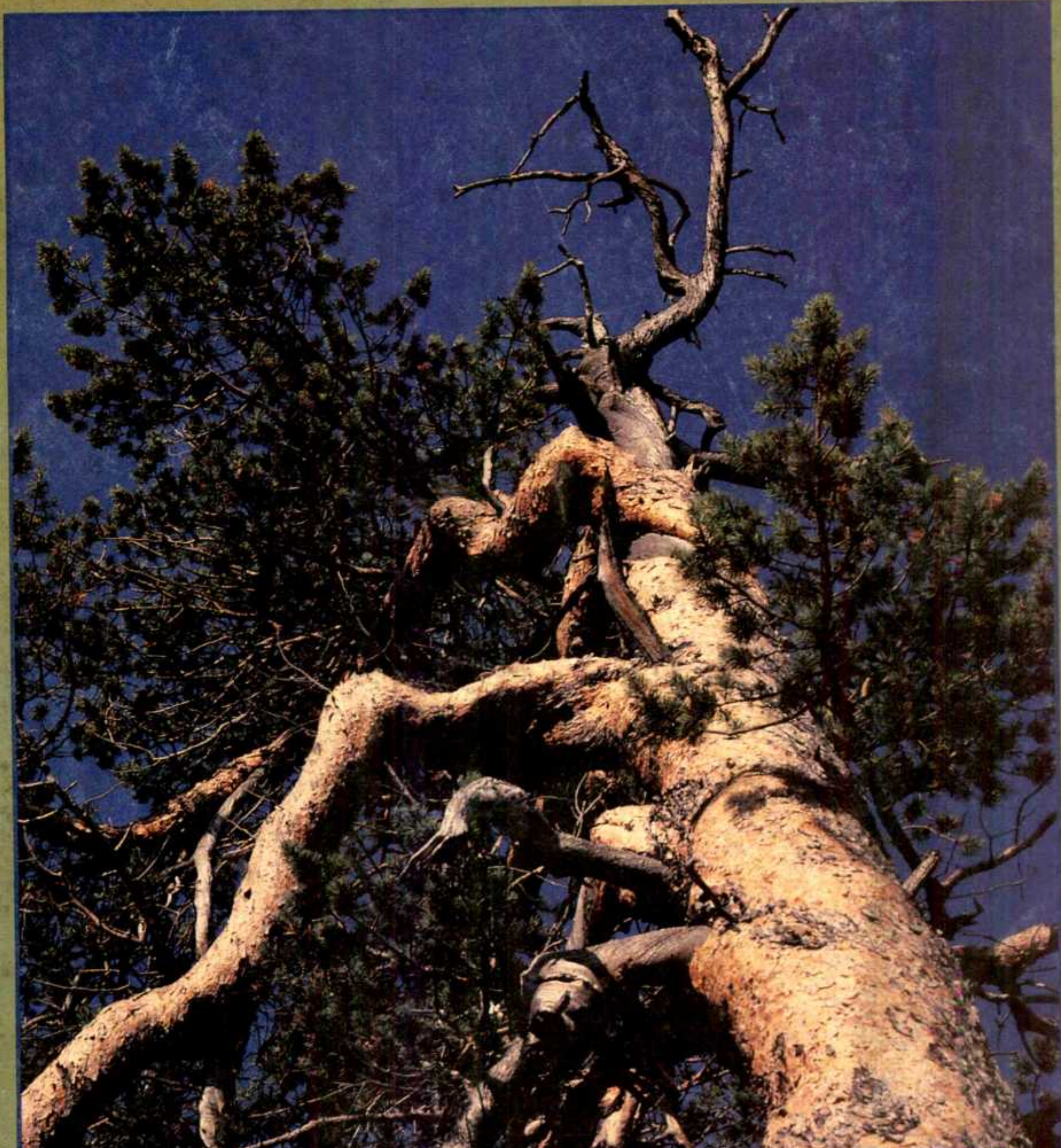
Volume 346 No.6283 2 August 1990 £1.95

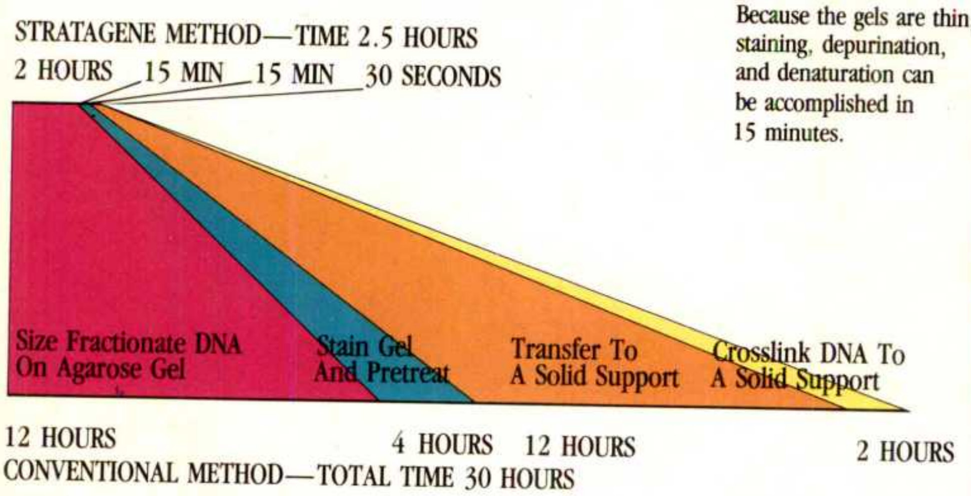


TREE RINGS AND CLIMATE

Stereoselectivity of beta particles
Adhesion receptors of the immune system

Southerns/Northerns: Electrophoresis, Blotting, and Crosslinking in 2.5 Hours Instead of 30

Stratagene has streamlined agarose gel electrophoresis and blotting. The system decreases the time required, from sample loading to prehybridization ten fold.



VAGE™ System

The VAGE™ vertical agarose/acrylamide gel electrophoresis system allows the casting of agarose or acrylamide gels in the unit. Nucleic acids can be electrophoresed through a 3 mm, 0.8% vertical agarose gel in less than two hours with excellent resolution (Figure 1). Because the gels are thin, staining, depurination, and denaturation can be accomplished in 15 minutes.

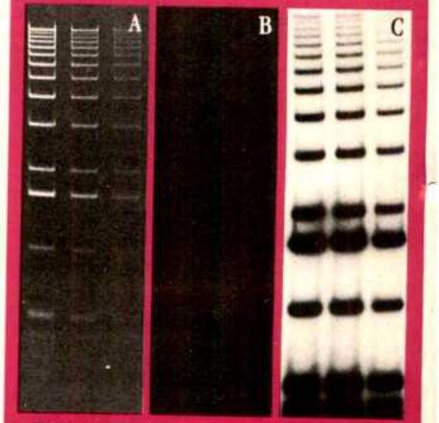
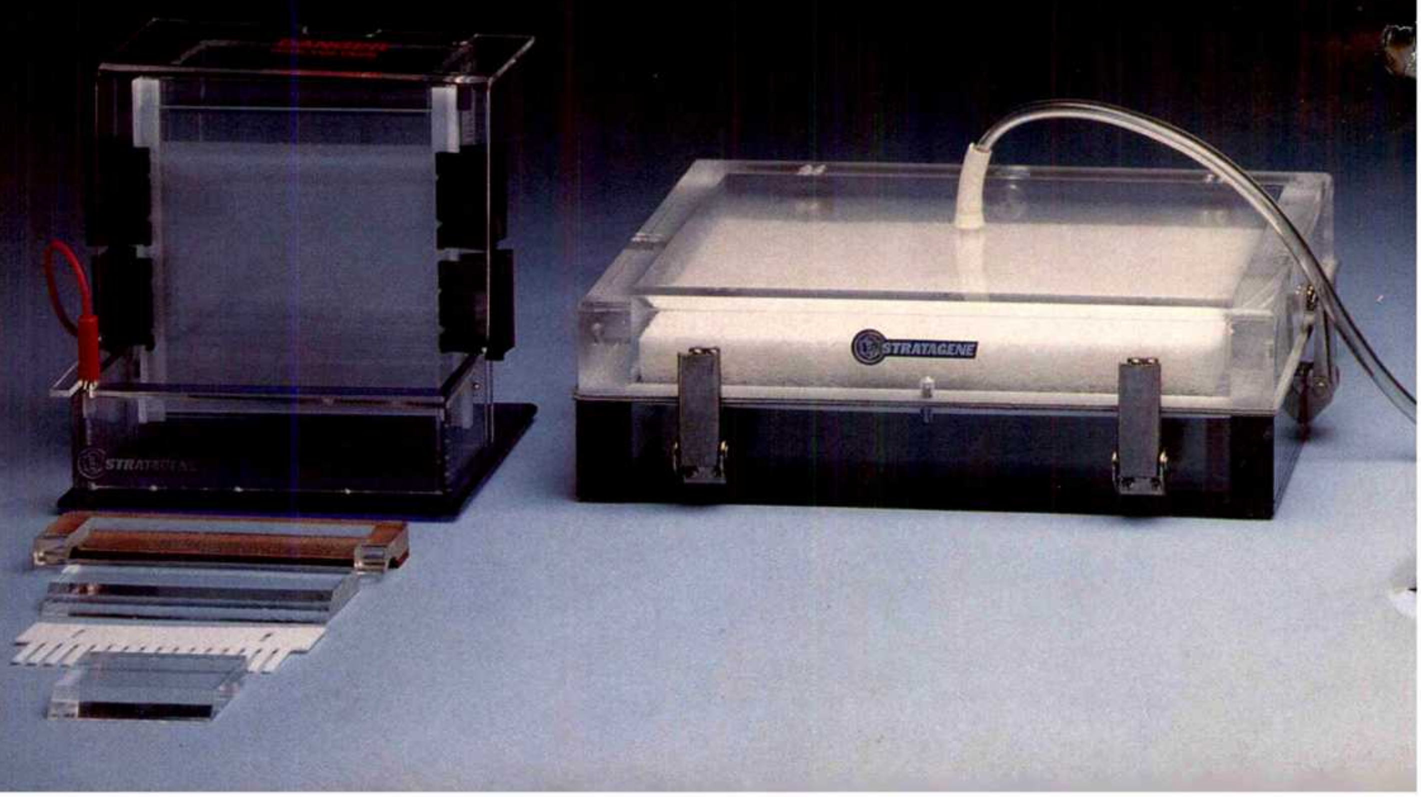


FIGURE 1:

Figure Legend: Fractionation of end labeled DNA markers on 3mm thick 0.8% agarose by the VAGE apparatus and transfer to Duralon—UV™ membranes using the PosiBlot pressure blotter.

- A. Ethidium stained gel showing high resolution.
- B. Same gel after pressure blotting.
- C. Autoradiogram of membrane after pressure transfer.



PosiBlot™* Pressure Blotter

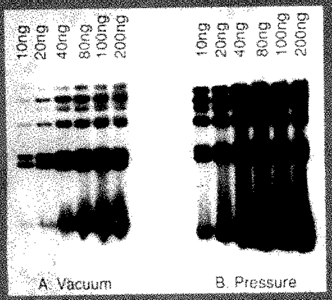


FIGURE 2:
Figure Legend: ^{32}P end-labeled lambda Hind III markers were electrophoresed in 0.8% agarose. The DNA was then transferred to a nylon membrane with a vacuum blotter at 30mm Hg below atmospheric or with the PosiBlot pressure blotter at 100mm Hg above atmospheric. Both transfers were carried out for 15 minutes. As can be seen, pressure blotting transferred significantly more DNA in the same period of time, especially in the higher molecular weight range (largest band is 23 kilobases).

The PosiBlot™ positive pressure blotter permits the transfer of nucleic acids in 1/3 the time of vacuum blotters and 1/50 the time of capillary blotting (Figure 2). Pressure blotting does not dehydrate gels as do other methods. This allows the use of substantially higher pressure differentials, compared with vacuum blotting, without gel collapse. The PosiBlot apparatus reduces blotting time to 15 minutes.

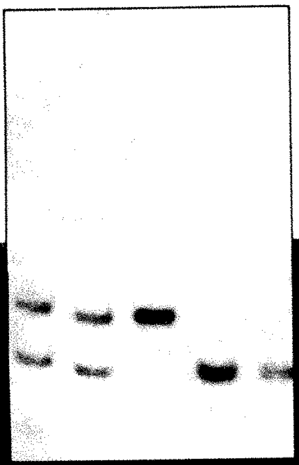


FIGURE 3:
Figure Legend: Autoradiogram showing the resolution of 2.8 and 1.3 Kb Msp I RFLP alleles revealed by a cystic fibrosis human DNA probe using the VAGE, PosiBlot and Stratalinker all in 2.5 hours.

Stratalinker™* UV Crosslinker

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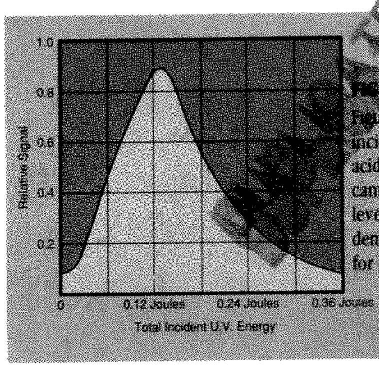

FIGURE 4:
Figure Legend: The effects of altering the incident energy for crosslinking nucleic acids to nylon membranes. The significant drop in signal intensity at energy levels below and above 0.12 Joules demonstrates the limited optimal range for UV treatment.

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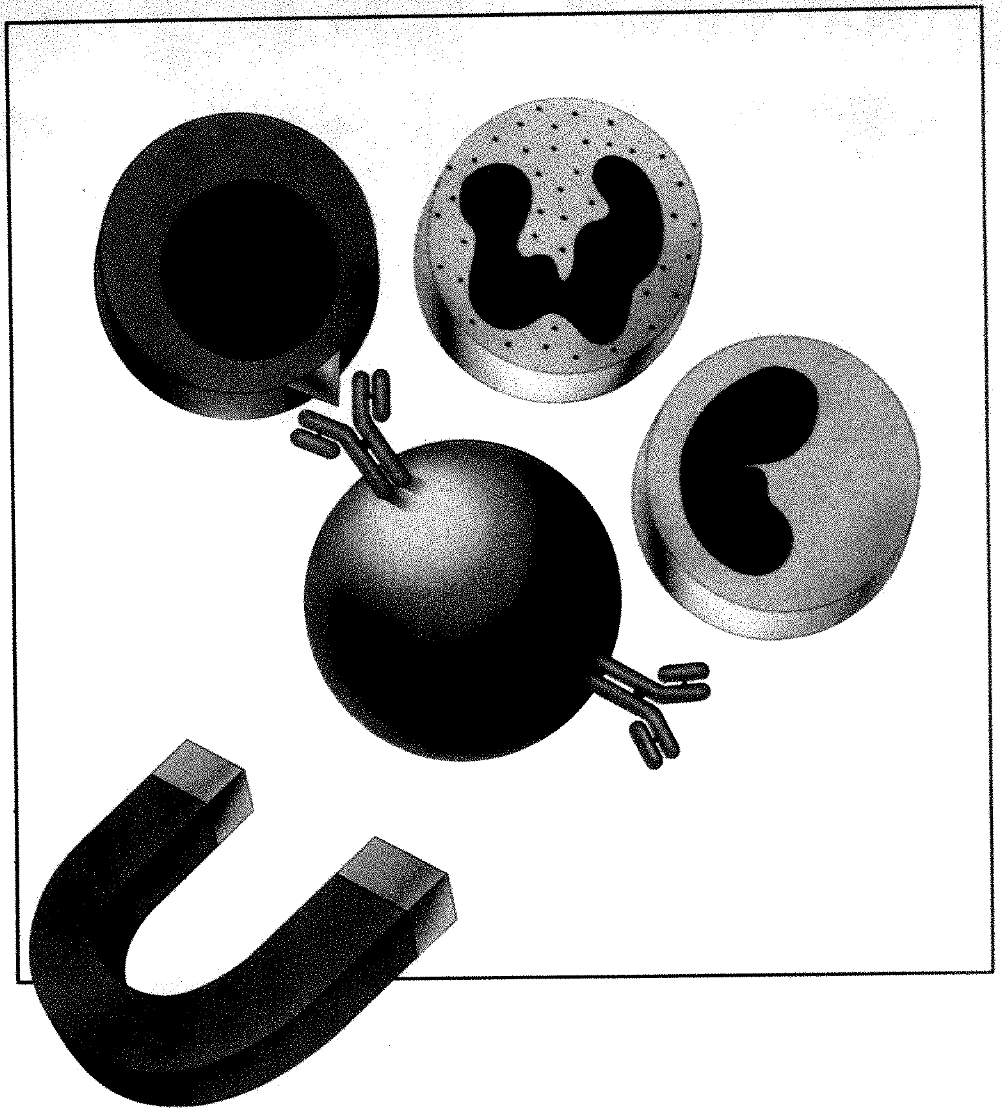

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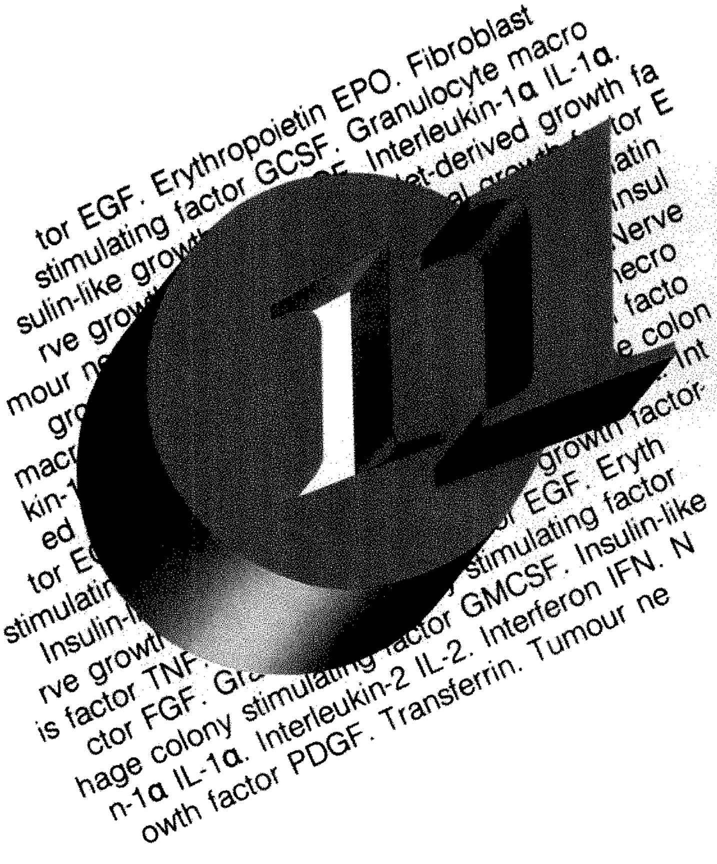
tor EGF. Erythropoietin EPO. Fibroblast
stimulating factor GCSF. Granulocyte macro
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growth factor GMCSF. Insulin-like
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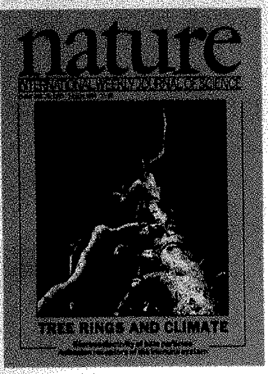


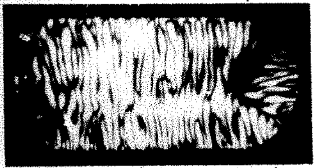
nature
2 August 1990
Vol. 346 Issue no. 6283

◀ A 600-year-old living Scots pine in the Torneträsk area of northern Sweden, a contributor towards perhaps the longest tree-ring record of climate so far obtained, covering nearly 1,500 years from AD 500 to the present. What were Scandinavian summers like long ago? For the answer, see page 434.

THIS WEEK . . . THIS WEEK . . . THIS WEEK . . .

Stereo systems

New experimental evidence suggests that when longitudinally spin-polarized electrons (beta particles) are passed through a liquid containing chiral enantiomers, they interact differently with each of the two mirror-image components of the liquid. The result may have implications for the origin of chirality in biological systems, page 451. In another *in vitro* study of relevance to the nature of living systems, solutions of the microtubule protein tubulin are shown to produce spatial structures (as above) in an energy-dissipative process that requires organic phosphate as an energy source, page 448.



Immune adhesion

The ability of cells of the immune system to patrol the bloodstream for infectious organisms, and on infection to aggregate in the lymph nodes and engage in specific cell–cell interactions that result in the elimination of the infectious organism, all depend upon closely regulated cell–cell and cell–substrate adhesive interactions. These are reviewed on page 425 by Timothy Springer.

China syndrome

Nasopharyngeal carcinoma is far more frequent among southern Chinese than any other population, and a linkage study suggests that this may in part be associated with a disease susceptibility gene linked to the HLA locus. Page 470.

Eggs unearthed

A recent rock fall in Transylvania has revealed the first dinosaur eggs to be found in Romania — *Magyarosaurus* eggs some 65 million years old. Page 417.

Wall game

Recent surveys of galactic redshifts provide evidence of large-scale organization in the Universe — Great Walls, voids and periodic structures — which are not easily explained by gravitational instability. A random cellular model overcomes some of these difficulties, page 446.

Cool for peptides

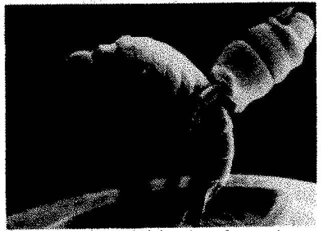
A mutant cell that normally fails to express class I molecules of the major histocompatibility complex on its surface can be induced to do so by incubation at low temperatures when the molecules will assemble on the cell surface in the absence of bound peptide. Page 476.

Cold front advances

The Last Glacial Maximum occurred about 20,000 years ago in northern Europe. But new evidence shows that in one region of France the furthest advance of the ice sheet occurred much earlier, between 30,000 and 50,000 years ago, and was greater in extent than was previously thought. Pages 453 and 410.

Uneasy rider

Pagothenia borchgrevinki — a species of marine invertebrate common in Antarctic waters — chemically defends itself against plankton-eating fish by abducting small individuals of an abundant pelagic pteropod species, one which is itself noxious to predators. See page 462.



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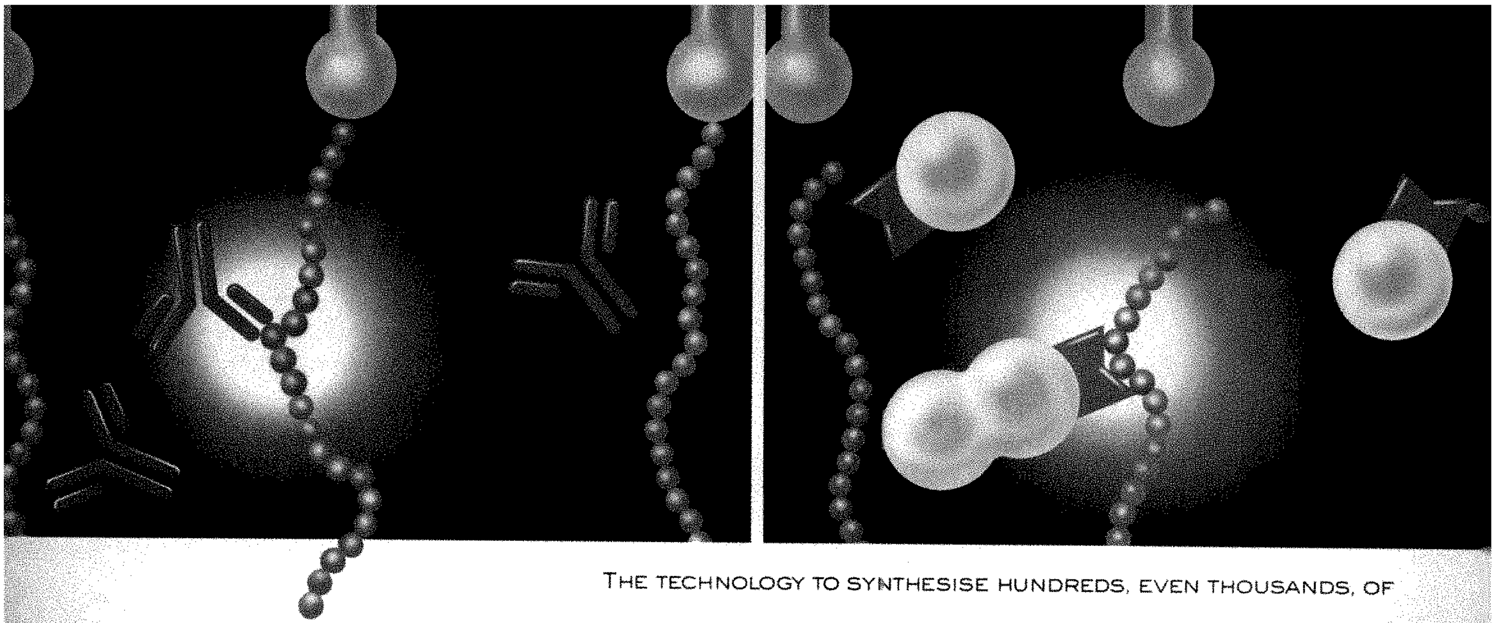

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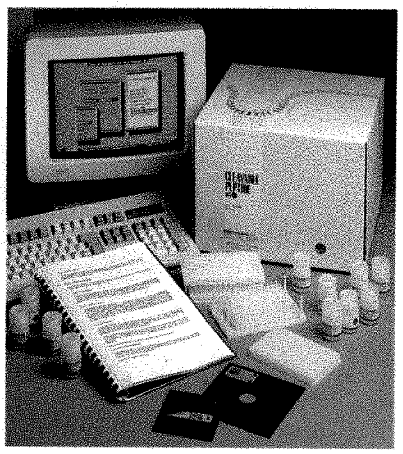

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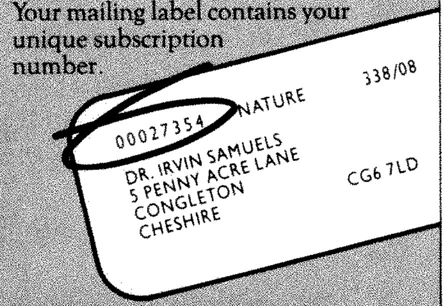

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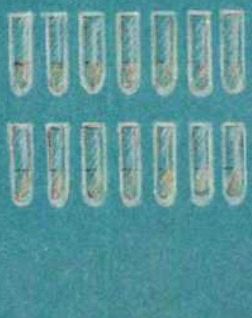

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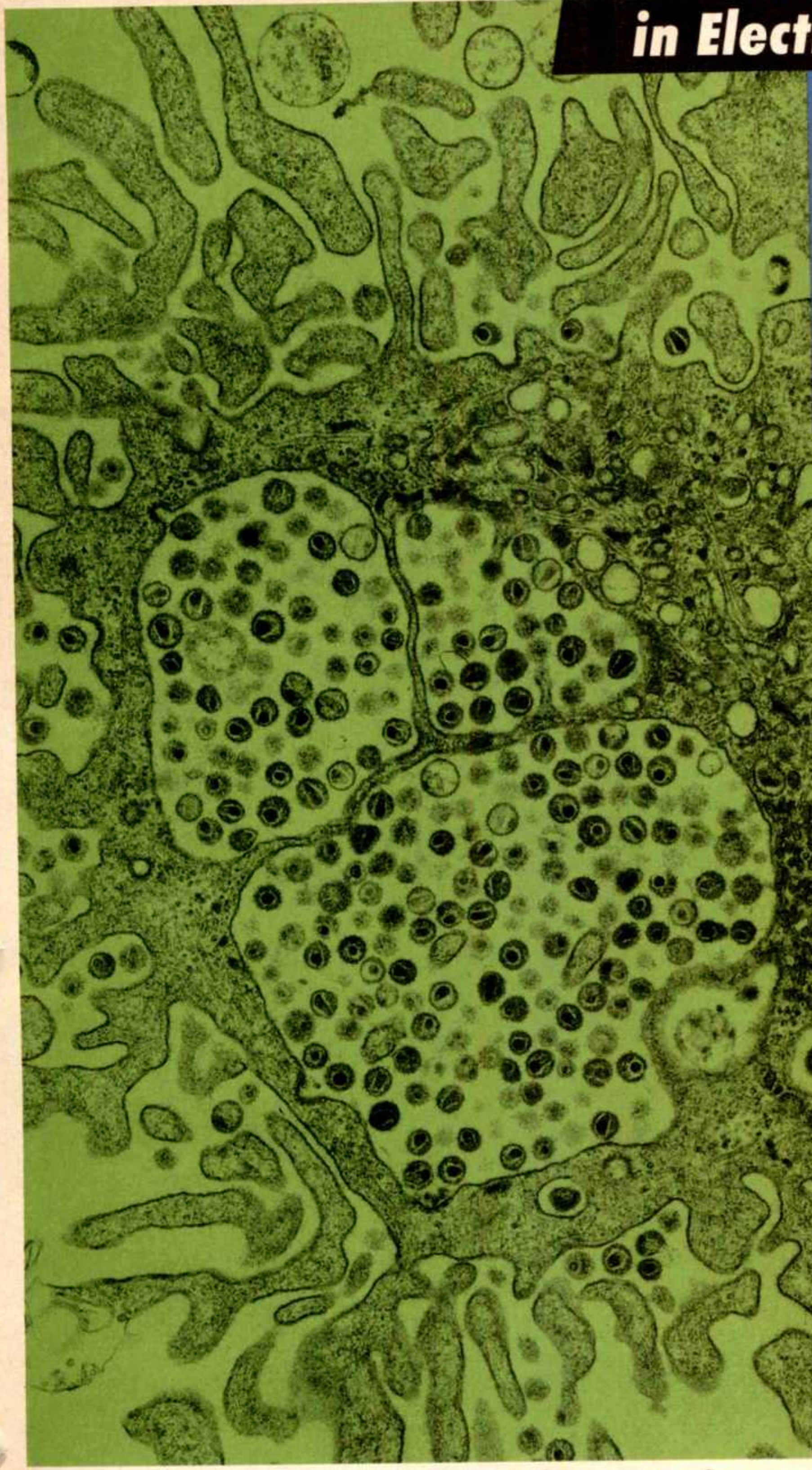

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Tuesday

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CHAIR: Dane Zabriskie, Director, Biological Process Science, SmithKline Beecham

FERMENTATION MONITORING: BIOSENSORS AND MASS SPECTROMETRY

On-line, real-time process monitoring sounds easy...and isn't. This is the reality.

CHAIR: Jerome Schultz, Director, Center for Biotechnology and Engineering, University of Pittsburgh

Wednesday

AFFINITY CHROMATOGRAPHY

Affinity separations in all formats, with an emphasis on process-scale.

CHAIR: Yves Fouron, Vice President, Business Development, Sepracor

TAKING CONTROL OF GLYCOSYLATION

We no longer have to settle for whatever glycosylation pattern a process produces...and it may be that the FDA won't let us. Learn how glycoprotein products are analyzed, remodeled, and may eventually be regulated.

CHAIR: Charles Goochee, Associate Professor, Stanford University

Thursday

CAPILLARY ELECTROPHORESIS

Last year's novelty has become a mainstay of laboratory and process analysis.

CHAIR: Andrew G. Ewing, Associate Professor of Chemistry, Pennsylvania State University

PROCESS VALIDATION: CURRENT CONCEPTS, FUTURE CHALLENGES

Industry and regulatory perspectives on validation - current questions and future challenges.

CHAIR: Anthony Lubiniecki, Vice President, Biopharmaceutical Manufacturing and Development, SmithKline Beecham

Product Discovery and Design

Tuesday

DESIGN FOR MANUFACTURING

Integrated facilities design including effective microbial containment, automation, and a customer's view of project commissioning.

CHAIR: Duncan Low, Director, Process Services, Process Separation Division, Pharmacia LKB Biotechnology AB

FINDING AND USING NEW ENZYMES FOR rDNA

Creating new, more specific DNA cleaving agents, important for both basic research and product discovery.

CHAIR: TBA

Wednesday

PROTEIN ENGINEERING

Fine-tuned pharmacology—via structural analysis and molecular mimickry—will be the foundation of vastly improved second- and third-generation biopharmaceuticals.

CHAIR: Gaetano Montelione, Center for Advanced Biotechnology and Medicine, Rutgers University

SCANNING-TUNNELING MICROSCOPY

The technique that gave us our first direct images of DNA molecules may develop into a fast alternative for sequencing the human genome.

CHAIR: Carlos Bustamente, Professor of Chemistry, University of New Mexico

Thursday

DIAGNOSTICS AND DEVICES: DEVELOPMENT AND REGULATION

In vivo imaging, infectious disease diagnostics, and gaining approval of new diagnostics as regulators tighten rules on "substantial equivalence".

CHAIRS: Jennifer Van Brunt, Managing Editor, Bio/Technology - Henry Miller, Special Assistant to the Commissioner, U.S. FDA

AGBIOTECH

The Plant and Animal
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Tuesday

AGRICULTURAL BIOTECHNOLOGY

Improved agronomics, from alternatives chemical pesticides to disease resistan in transgenics.

CHAIR: Robert Goodman, Executive Vice Pre dent, Research and Development, Calgene

TRANSGENIC PLANT PRODUCTS: CASE STUDIES

The companies making new markets agbiotech discuss product planning, d velopment and introduction; regulato and public acceptance.

CHAIR: Robert Goodman

Wednesday

MOLECULAR PHARMING: PLANTS AND ANIMALS AS BIOREACTORS

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PLANTS

Producing bioactive compounds, fro cytokines to human antibodies, in tran genic plants.

CHAIR: Andrew Hiatt, Department of Molecul Biology, The Research Institute of Scripps Clinic

ANIMALS

Engineering animals to produce pha macological compounds via controlle expression.

CHAIR: Katharine Gordon, Genzyme

Thursday

ENGINEERING LIVESTOCK

Embryo transfer and gene manipulatio for better breeds.

CHAIR: TBA

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Adding value through preservation an improvement: better table tomatoes, alte ing seed storage proteins, and improvin fish.

CHAIR: Indra Vasil, Professor, Dept. of Vegetabl Crops, University of Florida

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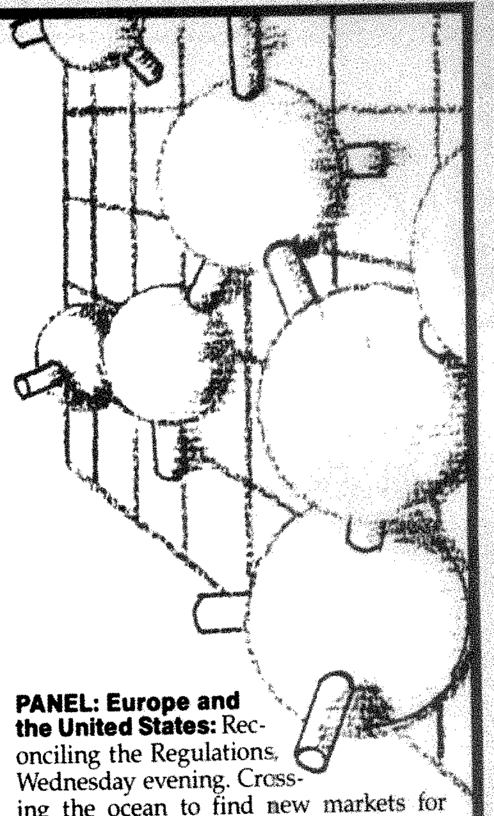

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lecting the right nucleic-acid and pro-in-blotting materials, reagents, and link-g methods.
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ENE AMPLIFICATION AND PCR
he latest applications from the origina-rs of polymerase chain reaction, one of e key techniques in molecular biology.
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dnesday

ENE MANIPULATION AND RANSFORMATION SYSTEMS
allistic injection of DNA-coated micro-arriers—a hot new approach for intro-ucing novel genetic material without amage.
HAIR: Vanessa Manges, DuPont

ULSED-FIELD ELECTROPHORESIS
lew programmable pulsed-field systems enerate any desired electric field pattern.
HAIR: Stephen D. Ferris, Bio-Rad

IPLC
ips on updating the lab workhorse to rovide faster, more consistent results.
HAIR: TBA

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SMALL SCALE CELL CULTURE
Iollow-fiber reactors improve on the tra-litional shaker flask.
HAIR: Ken Patti, Amicon

DNA REAGENTS AND TECHNIQUES
urgically precise restriction enzymes elp researchers save steps and get more neaningful results.
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BIOBUSINESS

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Tuesday

MARKETING AND REIMBURSEMENT ISSUES
Marketing veterans of successful biopharmaceutical product launches discuss strategic alliances, sales tactics, and insurance reimbursement.
CHAIR: Kathy Wiltsey, Director of Marketing, Amgen

ANIMAL RIGHTS: EFFECTS ON THE LABORATORY
The publishers of Animal Rights Reporter detail what to expect from the Animal Welfare Act. A panel on anti-activist tactics and facility security will follow.
CHAIR: Mary Lou Sapone, Perceptions Press

Wednesday

CONFLICT-OF-INTEREST REGULATIONS: THE PRACTICAL IMPACT
Though proposed regulations have been dropped, this issue won't go away. Could you or your company be vulnerable?
CHAIR: Steven Bongard, Director, Forum on Drug Development and Regulation, Institute of Medicine, National Academy of Sciences

CAREER DEVELOPMENT
How to find — and be — the right person in the right place for the fast track.
CHAIR: Janet Tweed, Principal, Gilbert Tweed Associates

Thursday

PATENT FOR THE TECHNICAL MANAGER: "INTELLECTUAL PROPERTY PRIMER"
Pointers on technology management in academic settings: what is worth protecting, how to balance the need to publish vs. the need to profit, and more.
CHAIR: John Wetherell, Attorney, Spensley, Horn, Jubas and Lubitz

LICENSING: OPPORTUNITIES AND SOUND PROCEDURES
Vantages of academia, corporations, and government examined, along with the tax implications of licensing technology.
CHAIR: John Wetherell

PANEL: Europe and the United States: Reconciling the Regulations, Wednesday evening. Crossing the ocean to find new markets for biotech products can be burdensome. Learn what's being done to harmonize the rules so that running the regulatory maze once will be enough.
CHAIR: Gregory C. Simon, Staff Director and Counsel, House Science, Space & Technology, Subcommittee on Investigation & Oversight

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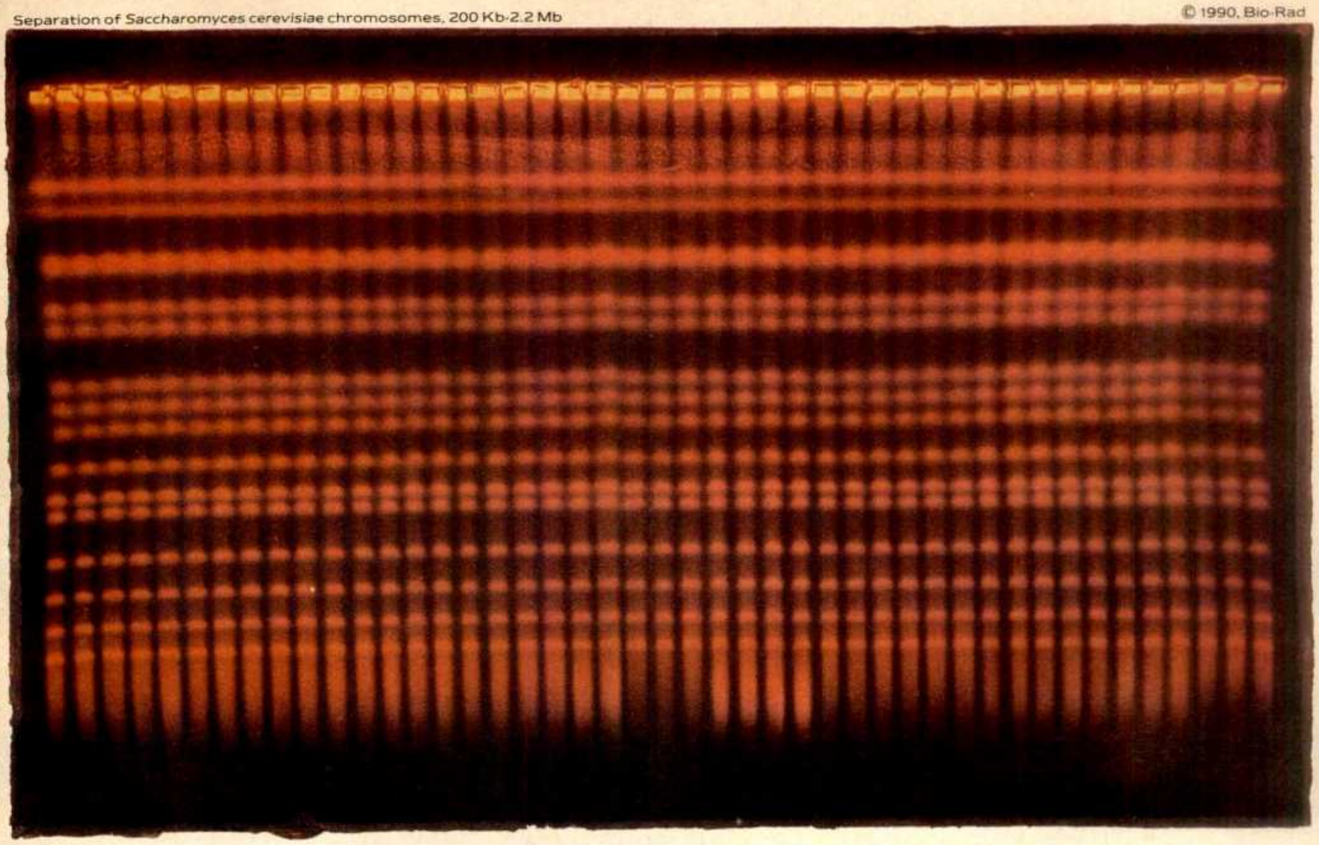

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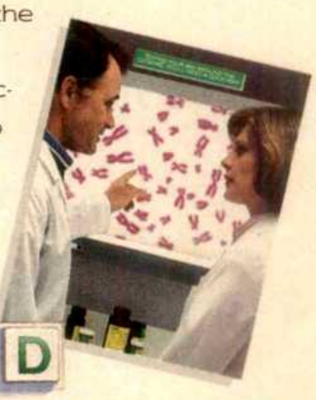

[1] D. C. Schwartz and C. R. Cantor. *Cell*, **37**, 67 (1984).
[2] Programmed Autonomously Controlled Electrodes. See S. M. Clark, E. Lai, and L. Hood. *Science*, **241**, 1203 (1988).

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Poster Session
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GENETIC DISEASE GENES I

KAY E. DAVIES
University of Oxford, Oxford
MOLECULAR ANALYSIS OF X-LINKED DISEASE

WEBSTER K. CAVENEE
Ludwig Institute for Cancer Research, Montreal
GENETICS OF GROWTH EXCESS DISORDERS
ASSOCIATED WITH TUMORS

Monday PM
Parallel Sessions
and
Award Lecture
(Evening)

DNA SEQUENCING

MICHAEL W. HUNKAPILLER
Applied Biosystems, Foster City
AUTOMATED DNA SEQUENCING

MATHIAS UHLEN
Royal Institute of Technology, Stockholm
STRATEGIES FOR SOLID PHASE DNA
SEQUENCING

VIRAL VECTORS

DAVID M. KURNIT
Howard Hughes Medical Institute, Ann Arbor
THE RECOMBINATION-BASED ASSAY:
ISOLATION OF CHROMOSOME-SPECIFIC
SEQUENCES

RICHARD C. MULLIGAN
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APPLICATIONS OF GENE TRANSFER
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MOLECULAR GENETICS OF CYSTIC FIBROSIS
AND NEUROFIBROMATOSIS

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Children's Hospital, Boston
MOLECULAR APPROACHES TO
NEUROMUSCULAR DISEASE

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& Plenary Session

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VICTOR A. McKUSICK
Johns Hopkins Hospital, Baltimore
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LOCALIZATION IN THE DIAGNOSIS AND
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Human Genome Center, Lawrence Berkeley
Laboratory, Berkeley
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HUMAN CHROMOSOMES

DAVID SCHLESSINGER
Washington University School of Medicine,
St. Louis
YEAST ARTIFICIAL CHROMOSOME BASED
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& Poster Session
(Evening)

COGENE ROUNDTABLE

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APPLICATIONS AND IMPLICATIONS

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Lawrence Livermore National Laboratory,
Livermore
THE CONSTRUCTION AND APPLICATION OF A
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Imperial Cancer Research Fund, London
LESSONS LEARNT FROM SEARCHING FOR
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DAVID WARD
Yale University, New Haven
ANALYSIS OF GENES AND CHROMOSOMES BY
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Plenary Session

GENETIC DISEASE GENES II

JAN L. BRESLOW
Rockefeller University, New York
GENETIC BASIS OF LIPOPROTEIN DISORDERS

DOUGLAS C. WALLACE
Emory University School of Medicine, Atlanta
MITOCHONDRIAL GENES AND
NEUROMUSCULAR DISEASE

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Award Lecture
and
Plenary Session

THE SPECIAL ACHIEVEMENT AWARD

PAUL BERG
Beckman Center, Stanford University, Stanford
THE ANALYSIS OF MOLECULAR GENETIC
PROCESSES VIA DESIGNER MINI
CHROMOSOMES

CANCER-RELATED GENES

DAVID E. HOUSEMAN
Massachusetts Institute of Technology, Cambridge
GENETICS OF WILMS TUMOR

ALFRED G. KNUDSON
Institute for Cancer Research, Philadelphia
HEREDITARY CANCER AND TUMOR
SUPPRESSOR GENES

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&
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HAIG H. KAZAZIAN, JR.
Johns Hopkins University, Baltimore
DIRECT GENE DIAGNOSIS IN PRENATAL &
PRESYMPTOMATIC TESTING

HENRY A. ERLICH
Cetus Corporation, Emeryville
GENETIC ANALYSIS USING PCR

ANIMAL MODELS

BARRY J. COOPER
New York State College, Ithaca
ANIMAL MODELS OF MUSCULAR DYSTROPHY

JOHN D. GEARHART
Johns Hopkins University, Baltimore
MOUSE MODELS THROUGH TRANSGENESIS

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W. FRENCH ANDERSON
National Heart Lung & Blood Institute, NIH,
Bethesda
THERAPEUTIC APPLICATIONS OF HUMAN
GENE TRANSFER

C. THOMAS CASKEY
Howard Hughes Medical Institute, Houston
MOLECULAR SCANNING FOR HUMAN DISEASE
MUTATIONS

THEODORE FRIEDMANN
University of California, San Diego, La Jolla
MODELS FOR HUMAN GENE THERAPY

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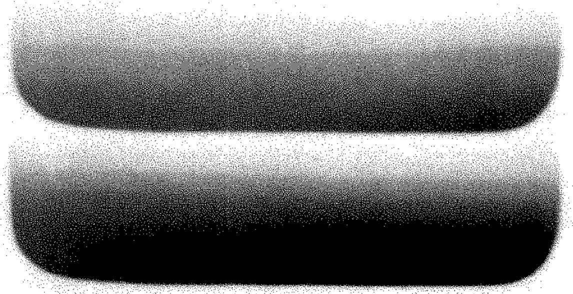

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Natural History Museum rumpus

The British Natural History Museum has taken too short and too jaundiced a view of its own future as a research institution. It should mend its ways, and quickly.

WHAT emerges most clearly from the long controversy about the future of the British Natural History Museum (NHM) is that the management's 'corporate plan', published in February, was a serious error of judgement. Faced with the prospect of a nasty financial squeeze — a shrinking budget and the high cost of maintaining the building in which it is housed — the museum plumped for the wrong solution, that of cutting back on an already inadequate intramural research programme. This is not the first time in the past ten years that British research institutions have been forced by financial pressure to respond in such a way. But usually there has been no choice. At the NHM, on the other hand, it would have been possible to cut back instead on the museum's second function of mounting attractive public exhibitions. The museum seems to have plumped for what it calls its "front-of-house" activities.

If the correspondence appearing in *Nature* is anything to judge by (see, for example, page 406), that reaching the museum directly has probably been even more blistering. It is all very well for Sir Walter Bodmer, the new chairman of the trustees, to imply that the volume of comment excited by the corporate plan is a measure of the regard in which NHM is held (*Nature* **345**, 569; 1990), but what is to be said when most of the comment is critical of the decision? What the rumpus has shown is that the rest of the world regards the NHM's collection as a unique resource, which it fears may now be lost to scholarship.

It is only fair to NHM to acknowledge that the fear may prove groundless. If the plan to separate curatorship from research succeeds as the museum intends, the collections may be no less accessible. The much more serious worry is that the reduction of the volume of research activity (by about a fifth) will further attenuate the museum's in-house expertise, compromising its capacity to act as a cheer-leader for taxonomy in Britain or anywhere. To say this does not imply that everything that used to happen at NHM was first-rate, but merely that relinquishing research activities is, in the present British climate, a recipe for permanent abandonment.

Worse still is the superficiality of the language of the corporate plan. The truncated research programme, which it is said will be "responsive to audience needs", has been renamed under headings such as Environmental Quality and Human Health. (In the approving digest of the corporate plan in last week's survey of British spending on research, "health" is at one place wishfully misprinted as "wealth".) The justification of the latter, according to the new director, Dr Neil Chalmers, is the museum's acknowledged expertise in the identification of the vectors of human diseases (*Nature* **345**, 198; 1990). But that is an unconvincing explanation for such a comprehensive title, however trendy. The "front-of-house" seems to have altogether too much influence on what little will in future happen back-stairs.

It is not as if the "front-of-house" will save the museum from the troubles that lie ahead. The temptations in this direction are of course natural; since the imposition of entrance charges, NHM has been collecting more than £2 million a year from its visitors. The hope is that spending more funds on better exhibitions will increase this source of revenue. But there is no chance that the extra income will pay for the cost of earning it, Yet the scale on which funds are to be spent on better exhibitions will not create a temple for the celebration of discovery to match, say, the new Musée de la Cité in Paris. Naturally, there is a chance that better exhibitions will win round some young people to careers in science. But does NHM know enough about its audience, and their susceptibility to exhibitions, to make that likelihood substantial?

What should happen now? There is no chance that the museum will change its mind. Its corporate plan is, in any case, already part of the British government's planning for the future. But even that unfortunate document acknowledges that the real crunch lies two or three years ahead. However buoyant the museum's bookshop and restaurant sales, the forecasts point to a danger that the scale of NHM's activity will be further under pressure two or three years from now. Should not the museum be making the case for relief from that further squeeze right now and — for the sake of its constituents — in public? It should also do more than has yet been done to show that there is substance in its hope that support for research by British academics at the museum will indeed be provided by the research councils, themselves not flush with funds. And if it is true, as museum officials have been saying, that the corporate plan was never intended to persuade the scientific community but only the Office of Arts and Libraries and, ultimately, the British Treasury, should it not now provide a more intelligent statement of its aspirations for the continuation of research at the museum? If the outcome should be a plan that cannot be financed out of the present budget, it would not be improper that the trustees should set out to find the funds from elsewhere. But this time, the museum should consult its constituency in advance, and not simply produce a document like a rabbit from a hat.

What NHM should have learned from the commotion of the past several weeks is that it is not just another British research institution, as disposable as Kleenex, but one whose research function is genuinely international. The museum should have been particularly stung by those who have donated their personal collections to the museum in the belief that it was a perpetual institution. NHM's collections and their investigation have a better claim on such a status than most other research collections. The trustees would now seem to have a stark choice: either to exert themselves so as to redress the balance between exhibition and research, if necessary making nuisances of themselves in the process, or to resolve to dispose of the collection to some institution elsewhere that will be able to use it properly. □

Computers for sale

The sale of ICL to a Japanese company is not necessarily a bad thing.

INTERNATIONAL Computers Limited (ICL), the British computer manufacturer sold this week to Fujitsu, has a chequered but absorbing history. It is the inheritor of the venture embarked on by the British company Ferranti in the late 1940s which led, by 1949, to the realization of an admired and then technically advanced digital computer. But the expectation that the machine would be the foundation of a successful British computer industry were frustrated by the peculiarly British expectation that ambitious technical developments can be sustained on a financial shoestring. Over the past four decades, the company has had several narrow escapes from catastrophe, but in the past decade has won itself an enviable reputation as an independent manufacturer of mainframe machines equipped with sophisticated software. The partnership with Fujitsu should at least keep the tradition alive.

So why should the rest of Europe be up in arms about the intended merger? The simplistic argument is that it is somehow disloyal to the concept of European unity that a strategically important manufacturer based in Europe should be controlled financially from outside. But that is a little European's view. Is it not far better that Europe should have a financially strong computer manufacturer, able to operate internationally, than that it should be saddled with an implicit obligation to protect ICL (and other European manufacturers) from outside competition? The planned merger, in short, should help to dissuade the new Europe from chauvinistic protectionism. The US Congress, about to embark on another such bout of chauvinism, would be well advised to follow suit. □

Garbage, garbage . . .

The US General Accounting Office, skilled at uncovering waste in public spending, has been beaten by garbage.

"WHAT has four wheels and fries?" quip the wags at the US General Accounting Office (GAO). "A multipurpose truck cross-hauling nonhazardous municipal solid waste and pre-prepared potato foodstuffs which, if a refrigerated unit, is in contradiction of the regulations issued by the Secretary of Transportation as instructed by the House of Representatives bill HR 3386" intone their diligent colleagues, who have spent the past nine months compiling a document that must surely rank among the most inconclusive government reports of all time.

Asked by the investigations and oversight subcommittee of the Committee on Public Works and Transportation to investigate the possibility that the practice of food/garbage cross-hauling — using the same vehicle to transport both components of the human equation — is a risk to health, the GAO has concluded that, "[w]hile federal health and food safety experts contend that the risk of food contamination from cross-hauling with garbage is relatively low . . . [w]e, along with the federal regulators and health experts, believe that current information is not adequate to rule out health risks in transporting food in these trucks". In short, the GAO report is as noncommittal as its health experts.

Each of the document's 44 pages offers a new twist in what is obviously a cunning pastiche of a scientific report. The cover sets the tone: what better title for a groundbreaking report on possible health risks than *Truck Transport: Little Is Known About Hauling Garbage and Food in the Same Vehicles*? Similarly, chapter three — the report's thundering denouement — is simply entitled "Experts Do Not Know Potential Food Contamination Risks From Cross-Hauling Garbage and Food".

But it is all too easy to underestimate the task facing the compilers of the GAO report. At the mysterious request of state officials, two of the four landfill sites at the focus of the survey were out-of-bounds to the researchers. Yet, rather than cravenly finding two other suitable landfills instead, the intrepid accountants persevered, scribbling down the licence numbers of trucks using the off-limits sites. Although they were unable to speak to either the drivers or the operators, the GAO was ultimately successful in taking a photograph of a signpost in the road outside one of these sites, an achievement described triumphantly as "Figure 2.1: Sign Outside Ohio Landfill".

Another classic example of the visual aid is Figure 3.1, which does its best to justify the report's tenuous initial claim that "transporting food in a truck that previously hauled garbage inspires high emotions in many individuals". The accompanying photograph shows a truck full of garbage, allowing the document to reach its only conclusion: "As figure 3.1 depicts, a truckload of loose garbage is a disgusting sight". □

Safety in numbers for Earth Observing System

■ Industry report offers redesign
■ NASA decries unwanted advice

Washington

OFFICIALS at the National Aeronautics and Space Administration (NASA) last week attacked a draft report that suggests the space agency may be pursuing a poor design for its planned $40,000 million series of climate monitoring satellites, collectively known as the Earth Observing System (EOS).

The report, by three researchers from the space and technology group of California-based TRW Inc., argues that it would be better to construct EOS as orbiting groups of many small, relatively inexpensive satellites, rather than as a few large, multi-purpose platforms, each the size, complexity and cost of the Hubble Space Telescope.

The recent demoralizing failure of the Hubble's optics, along with suggestions that the proposed space station Freedom will require an impossible number of man-hours for routine maintenance, has added weight to the criticism that large, complex systems invite disaster. But Ray Roberts, EOS programme director, said that the unsolicited advice from TRW, which at present has no major contracts for EOS, reflected "vested interests". He warned against "opportunists" and "NASA-bashing" in the light of NASA's recent problems.

NASA's plans for EOS put as many as 16 different instruments on each of six large platforms to allow simultaneous observations of regions of interest. But a number of scientists, including James Hansen, director of the Goddard Institute for Space Studies, and members of a American Institute of Aeronautics and Astronautics (AIAA) review panel, have called on NASA officials to rethink the EOS design, arguing that the same scientific results can be achieved more reliably with the tightly-grouped "clusters" of specialized satellites similar to what the TRW report proposes.

The biggest concern is the possibility of a total loss of one of the proposed EOS platforms. All six are to be carried into polar orbits by relatively untried Titan IV rockets, whose predecessor, the Titan III, has suffered failures of varying degree in more than half of its launches over the past five years (see *Nature* **344**, 281; 1990). If EOS were to be built around smaller satellites, they would probably be launched from more reliable Atlas or Delta boosters, NASA officials say.

A breakdown in a critical power, control or data-transmission component of the EOS platforms could render all the on-board scientific instruments useless, critics point out. Shifting the emphasis to small satellites "alleviates the danger of placing all eggs in one basket [and] the possibility of losing all data from one launch or subsystem failure", Hansen writes in a paper scheduled to appear in *Issues in Science and Technology* this autumn.

With an orbiting group of several simple satellites, even the total failure of any one probe would be likely to result in no more than a partial loss of coverage. Small replacement satellites can be built relatively cheaply and quickly, the AIAA panel noted in a report released last month.

Other critics stress the danger of magnetic, electrical and thermal interference between sensitive instruments on a shared platform. And only with small satellites can researchers launch small packages of instruments to take advantage of new technology or to focus on a new area of research. Both Hansen and the AIAA panel note, for example, that because the EOS platforms will be restricted to polar orbits, they will be restricted in their ability to monitor hour-by-hour variations in clouds — a critical deficiency if clouds turn out to be as important to the global warming equation as is now believed. Indeed, Hansen's chief proposal is for additional satellites in both polar and inclined orbits to maximize coverage.

The TRW report says that many of the planned EOS instruments could make simultaneous measurements from small satellites orbiting in formation and separated by distances of three to five kilometres. TRW researchers Carl Graves, Peter Gottlieb and Alan Rosen decided that 24 main instruments of the type proposed for the first three EOS platforms near the end of the decade could be distributed on 17 smaller satellites in several clusters without loss of accuracy. The relative positions of the satellites could be maintained with only small daily adjustments, Rosen says. Because the satellites within a cluster are so closely grouped, they would be able to observe the same location on the ground within a half second of each other, which allows effectively simultaneous measurements even under rapidly changing weather conditions.

The TRW scientists are scheduled to release their report at a meeting of the International Astronautical Federation in October in Dresden, West Germany.

Shelby Tilford, director of NASA's earth science and applications division which runs the EOS programme, says that a switch to small satellites at this point would constitute a "new start" and could mean as much as a four-year delay in the project. A 1989 NASA study estimated that small satellites would cost 10–20 per cent ($4,000–$8,000 million) more than the planned large platforms. But the AIAA report warns that "the theoretical cost advantage claimed for large programs typically turns out, in practice, not to be realized because the amount of coordination . . . they require seems always to exceed the original projections". Big systems tend to be clumsy", it adds.

In April, an interim report from the National Academy of Sciences recommended that NASA investigate further the possibility of multiple small satellites as an alternative to its current EOS plans, at least for the second half of the project (see *Nature* **344**, 578; 1990). Panel members say that the final version of the report, due out later this year, will conclude that NASA's arguments for three large platforms for the first half of the programme are "overwhelming", but that technical reasons for large — rather than small — platforms for EOS's second half are much weaker.

G. Christopher Anderson

FOSSIL REPTILE

Lizzie home safe

London

LIZZIE, the fossil of the earliest-known reptile (see *Nature* 342, 676; 1989), now has a secure home at the National Museums of Scotland. The announcement comes after the successful conclusion of an appeal to prevent the Scottish fossil from being sold by its discoverer, Stan Wood, to the Museum für Naturkunde in Stuttgart.

Wood and the Stuttgart museum had agreed to suspend the sale until 31 July in order to give the National Museums of Scotland a chance to raise the £205,000 purchase price. A last-minute donation of £40,360 by the National Heritage Memorial Fund and another £20,000 from West Lothian District Council made up the price, following the lead set earlier this year by the £10,500 contribution from the Geologist's Association which prompted Wood to drop the price by the same amount (see *Nature* 345, 99; 10 May 1990).

This intensive wheeling and dealing, unprecedented in the quiet world of vertebrate palaeontology, has now reached a satisfactory conclusion that will allow Lizzie to be given a formal geological name. The proposal is *Westlothiana curryi*, in recognition of the fossil's place of origin and the part played by West Lothian District Council and the Curry fund of the Geologist's Association in securing the fossil for Scotland. Henry Gee

SPACE PHYSICS

Lighting up the sky

Washington

AFTER several delays due to technical problems, bad weather and more technical problems, the Combined Release and Radiation Effects Satellite (CRRES) was launched on 24 July by an Atlas-1 rocket from Cape Canaveral, Florida. From a highly elliptical orbit, CRRES will, over the course of a year, release puffs of fluorescent ionized gas at altitudes ranging from 240 to 21,000 miles, allowing scientists on Earth to study the structure and dynamics of the Earth's magnetic field.

The launch itself marked an important change of style for the National Aeronautics and Space Administration (NASA): instead of buying the rocket and launching it, NASA contracted out the task of putting CRRES into orbit to General Dynamics, the manufacturer of the Atlas-1. Efforts to create a commercial space business in the United States were begun by the Reagan administration after the Challenger explosion, but the first commercial launch was not until last year, when McDonnell Douglas used one of its Delta rockets to put a British communications satellite into geostationary orbit (see *Nature* **341**, 5; 1989). In the future, NASA intends that scientific satellites too should be launched by commercial arrangement with the various US rocket manufacturers.

The purpose of CRRES is literally to illuminate the geometry of the Earth's magnetic field in the upper atmosphere and near space. On board are 24 canisters, each containing about 10 kg of barium, calcium, lithium or strontium along with a small charge of boron and titanium, which, once ignited, burns spontaneously to vaporize the metals and create a cloud of ionized atoms.

Fluorescing under the action of ultraviolet radiation from the Sun, the glowing ion clouds will move along magnetic field lines, betraying their structure for the benefit of researchers.

By virtue of its elliptical orbit, CRRES will be able to release tracers into both the ionosphere, the ionized upper portion of the Earth's atmosphere, and the magnetosphere, the region beyond the atmosphere that is occupied by energetic charged particles supplied by the solar wind.

Both the ionosphere and the magnetosphere are regularly disturbed by outbursts of charged particles from solar flares, disrupting radio transmissions around the Earth. These phenomena have indirectly revealed something of the physical characteristics of the ionosphere and magnetosphere, but, by allowing experiments under controlled conditions, CRRES will be able to paint a more exact picture of these regions.

In addition to its payload of chemical bombs, CRRES also carries a number of instruments supplied by the Department of Defense to measure electromagnetic activity and particle densities in the vicinity of the satellite. These devices will be used to help choose the best moment to release the canisters, and to measure the properties of the ion clouds produced.

According to Gene Wescott of the University of Alaska, a principal investigator on several of the CRRES experiments, the ion clouds created in low-altitude releases should be visible to the naked eye for periods of many minutes. Inhabitants of the Caribbean island of Puerto Rico are likely to get the best show: in June and July next year, low-altitude CRRES experiments will be augmented by simultaneous releases of ion clouds from sounding rockets.

In some of these experiments, a mixture of barium, calcium and strontium will be released; their different masses and ionization energies make their interaction with the ionosphere visibly different in ways that will diagnose the complex properties of the plasma.

Once the ion clouds disperse and dim, it will take months, perhaps years, for the barium and other metals to descend to the surface. And environmentalists need not be alarmed. The tens of kilograms of barium aboard CRRES are far outweighed by the many tons that drop into the Earth's atmosphere every year in the form of micrometeorites.

David Lindley

Japan's angel tours the sky

Tokyo

ON Saturday, 4 August, Japan's first lunar probe Hiten (named after a Buddhist angel) will flash past the Moon in one of a rapid succession of lunar passes which, if successful, will send the tiny satellite hurtling off on its furthest venture yet into space. The probe will then return in March for some risky 'aerobrake' experiments in the Earth's upper atmosphere.

At 182 kg, Hiten weighs no more than a Japanese Sumo wrestler. But by cleverly exploiting the gravitational fields of the Earth, Moon and Sun, engineers at the Institute of Space and Astronautical Science (ISAS) hope to make Hiten perform a complex series of loops around the Earth and Moon without completely expending the probe's very limited stores of fuel.

Launched by a small solid-fuel rocket in January, Hiten was pulled out of an elliptical Earth orbit in March by the gravity of the approaching Moon. As it swung by the Moon, Hiten dropped a mini-satellite about the size of a basketball into lunar orbit before proceeding into a large elliptical orbit around the Earth extending about 750,000 km into space.

After nearly four months of leisurely Earth orbits, Hiten again passed close by the Moon on 10 July and was pulled into a tighter Earth orbit, with an apogee of 560,000 km, that brings the probe past the Moon every month.

Following three more lunar passes on 4 August, 7 September and 2 October, the Moon will sling the probe off into space, sending it out to a distance of 1,350,000 km from Earth on 17 November before the Moon catches it again early next year and brings it back into a tighter Earth orbit.

Another series of close encounters with the Moon will then force Hiten into an orbit that will bring it careering through the Earth's upper atmosphere at a height of only 120 km in mid-March 1991. ISAS engineers then hope to use the braking effects of the Earth's upper atmosphere to bring the high-speed Hiten back under control.

Jun Nishimura, director general of ISAS, says that if the probe survives its ride through the atmosphere it may then be placed at one of the zero-gravity 'Lagrange points', where the gravitational forces due to the Earth and the Sun cancel each other. It will be allowed to just "sit for a while", says Nishimura, after which Hiten's last drops of fuel may be used to push it into a lunar orbit alongside the mini-satellite released in March.

The small lunar orbiter has been the only disappointment of an otherwise successful mission. Shortly before the orbiter was released into lunar orbit, it was realised that the telemetry system of the orbiter was not working, so that ISAS engineers could not track it.

ISAS called on astronomers throughout Japan to train their telescopes on the Moon on 18 March, when the lunar orbiter's tiny engine was fired after it had been released by Hiten. A plume from the rocket was indeed photographed with a telescope on Mount Kiso, 200 km west of Tokyo, and although the lunar orbiter has never been seen or heard of since, ISAS scientists are confident that it successfully achieved lunar orbit.

But it is still uncertain whether Hiten will eventually join the lunar orbiter. Project manager K. T. Uesugi says the aerobrake experiments in the Earth's upper atmosphere next March are "quite risky" and may turn out to be the" end of Hiten's mission".

David Swinbanks

SUPERCONDUCTING SUPER COLLIDER

The sky is the limit

Washington

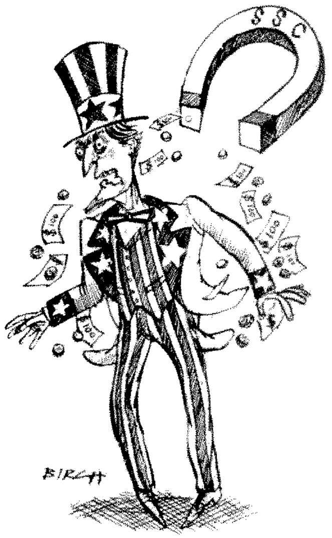
How much is too much for the Superconducting Super Collider (SSC)? At an estimated $8,000 million earlier this year, the planned particle accelerator's cost was already larger than the Gross National Product of two-thirds of the world's nations, the entire US yearly spending on health research and the cost of all current European particle accelerators combined. Now a Department of Energy (DOE) advisory panel says that the project will need still more — difficulties with the SSC's magnets and the extra costs of any delays in obtaining funds from Congress may drive the total price to $8,600 million by its completion in 1998, the panel said last week.

The SSC cost estimate panel, comprising members of DOE's High Energy Physics Advisory Panel (HEPAP), was the most pessimistic of three groups that have re-examined the project's financial prospects for DOE. An internal team of DOE experts puts the cost at $8,300 million. And an estimate by University Research Associates (URA), the contractor which will actually build the SSC, promises to finish the project at the bargain price of $7,840 million. A review by a fourth group, made up of outside financial experts, is still uncompleted. DOE will compare all four estimates and submit a final report to Congress on 17 August.

The chief concern seems to be the schedule and technical challenges of building the superconducting magnets. "The project has been planned on an extremely optimistic schedule, with every event being 'head-to-tail' with others . . . [S]uch an approach results in an unrealistically low estimate, which will inevitably be overrun", HEPAP warned. The panel also noted that the SSC has had difficulty attracting staff.

Beyond technical problems looms the very real possibility that Congress, squeezed by deficit reduction and still uneasy about the scientific arguments behind a 54-mile superconducting accelerator, may cut the project's funding at some time during the next eight years. Moore said that a congressional funding delay could add over $50 million a month to the SSC's final cost. The HEPAP panel says that schedule delays are likely to contribute $300 million to the overrun.

HEPAP also recommended that another $300 million — which would raise the total cost to $3,900 million — should be budgeted to build two large detectors that are part of the original SSC plan. But rather than raise the cost further, SSC officials have decided to drop the two large detectors for the time being. Two less expensive detectors — one medium, the other small — should be sufficient for the early years, says acting project manager Ted Kozman. He says that there may not be enough physicists available to justify the operation of larger detectors when the SSC begins operation at the end of the decade. Expansion room will be built into the SSC to allow larger detectors to be installed later when the demand rises, Kozman says.



Speaking at a press conference last week, DOE deputy secretary W. Henson Moore said that the department intends to go to Congress with the URA figures, rather than the higher estimates. "We're going to hold the contractor to $7.8 billion and to finish by 1998", he said. Although there is no penalty if URA is then forced to raise the cost, Moore said the contractor would have to document the need and get DOE approval. Submitting one of the higher estimates to Congress, Moore said, would only be sending the signal that congressional and technical delays are anticipated and compensated for. "If we were to take any other position, we'd be telling the contractor, 'We expect delays, don't worry about it'," he said. So far this year, Congress has treated the project relatively well, granting the full DOE request of $318 million for 1991. But until legislators trim spending across the board later this year to reduce the federal deficit, final budgets for all projects — including the SSC — will remain at risk.

Moore also announced the release of the formal request for proposals to build the superconducting magnets. Because the magnets will represent a substantial advance in technology and engineering, the companies or countries that provide them stand to become world leaders in the fledgling superconductor industry. Although DOE's offer stipulates that at least 50 per cent of the contract must go to US industry, companies from foreign countries may bid on the other half of the contract, perhaps as part of an 'in kind' contribution from their nation's government. So far, only Korea and India have expressed interest in contributing to the project. **G. Christopher Anderson**

ANTARCTIC RESEARCH

Keeping it clean for science

São Paulo

THE twenty-first meeting of the Scientific Organization on Antarctic Research (SCAR) ended here last week with the addition of four new full members — Spain, South Korea, Finland and Holland — and a new associate member — Colombia — bringing the total membership to 29 countries.

The scientific focus of the meeting was the effort to integrate Antarctic research with global scientific concerns, particularly climate change. The threat to the region's environment was also a theme of the meeting: "The environment is not one major issue, it is *the* major issue", said Brazilian geologist Antonio Carlos Rocha-Campos, executive secretary of SCAR and chairman of the meeting.

Dealing with the waste produced by an Antarctic population that can reach 4,000 in the summer months (December to March) has become one of the greatest environmental concerns. SCAR has produced a rulebook on waste management, but enforcing it is another matter. Not all of the 50 Antarctic stations have the requisite waste-disposal technology, but Rocha-Campos diplomatically refused to name the worst offenders. Inspections conducted by Greenpeace, however, have documented open-air trash burning at the Chinese, Chilean, Argentine, Uruguayan, US and Soviet bases.

Tourism is also becoming a headache, both as an additional source of pollution and because of disruption to the scientific activities of the stations. SCAR, an advisory body to the Antarctic Treaty nations which coordinate research efforts, considers tourism a "legitimate activity" when properly ordered, but scientists are finding it troublesome.

SCAR has no power to enforce its recommendations but has, for example, suggested that unnecessary duplication of activities should be avoided, especially in crowded areas such as the Antarctic Peninsula and the nearby King George Island. Crowding exacerbates environmental problems, but the mood of the SCAR meeting was that it is the responsibility of scientists to deal with problems that will ultimately disrupt the science they have come to Antarctica to conduct.

Ricardo Bonalume Neto

HUMAN GENE THERAPY

First experiment approved

Washington

AFTER bringing in their colleague Claudio Bordignon from Milan, Italy, to present the results of animal tests, R. Michael Blacse and W. French Anderson of the National Institutes of Health (NIH) have succeeded in convincing a subcommittee of the NIH Recombinant DNA Advisory Committee (RAC) that the time is right to go ahead with the first human gene therapy experiment. The researchers hope to treat children with severe combined immunodeficiency caused by ADA (adenosine deaminase) deficiency — a rare inherited immune disorder affecting fewer than 20 people worldwide — and although the experiment must still be approved by the RAC, the director of NIH and the US Food and Drug Administration, Anderson is optimistic that clinical trials in four children could begin as early as mid-autumn.

With increasing numbers of experiments being submitted for approval — there are four in the pipeline — there is concern that the review system will not be able to cope. The subcommittee is trying to carry out "a very critical sentence by sentence review", says Anderson, who feels that "this depth of review is not necessary".

All the new proposals awaiting review by the subcommittee are based upon the first gene transfer experiment, approved in January 1989 (see *Nature* **337**, 294; 1989), which was designed to trace the movement of tumour-infiltrating lymphocytes (TIL) in terminally ill cancer patients. The experiment was very controversial when first proposed, but has since formed the cornerstone of all new gene transfer and gene therapy proposals. In what are referred to as the N2-TIL experiments, Steven Rosenberg of the National Cancer Institute (NCI) isolated TIL cells from a patient's tumour, marked them with a gene for neomycin resistance and readministered them to the patient. Although the gene conferred no therapeutic benefit on the patient, it allowed researchers to track the path of TIL cells in the patient's body and determine whether the cells 'homed' to the tumour to fight the cancer.

With the safety of the N2-TIL gene transfer established, Blacse, of the NCI, and Anderson, of the National Heart, Lung and Blood Institute, plan to move on the "gene therapy" by inserting genes of therapeutic value into children who suffer from ADA deficiency. Using the methods of the N2-TIL experiments, but substituting the patient's own T lymphocytes for TIL cells, researchers hope to correct the ADA deficiency by inserting a normal human ADA gene into the T lymphocytes and returning the gene-corrected cells to the patient.

Of the four new experiments in the pipeline, two almost identical gene transfer experiments have been submitted by K. Brenner of St Jude's Children's Research Hospital in Memphis, Tennessee, and K. Cornetta of the University of Wisconsin. Brenner and Cornetta hope to determine the mechanism of bone marrow reconstitution following autologous bone marrow transplantation (ABMT), in which the patient's own bone marrow is used to treat leukaemias and some solid tumours. Marrow cells can be marked using the N2-TIL gene transfer system, and tracked when returned to the patient.

Building on his earlier N2-TIL experiments, Rosenberg is hoping to improve the anti-cancer properties of TIL cells by inserting a gene coding for tumour necrosis factor (TNF) — an experimental anti-cancer drug — into TIL cells. In a similar vein, M. T. Lotze of the University of Pittsburgh is looking at ways to improve the targeting and efficacy of TIL cells in patients with malignant melanoma. Performing an identical experiment to the N2-TIL gene transfer study, Lotze plans to study the effects of administering Il-2 and Il-4 in conjunction with TILs.

Diane Gershon

PEST CONTROL

Cleverer ways to kill?

Washington

ATTEMPTS by Western nations to combat the huge plague of locusts and grasshoppers that struck Africa in the late 1980s relied far too much on brawn and far too little on brains, according to an unusually critical report* released from the US Congressional Office of Technology Assessment (OTA) earlier this week.

In a massive campaign led by the US Agency for International Development (AID), some 13 million litres of insecticide were sprayed over 100,000 square kilometres of northwest and northern Africa between 1986 and 1989. As a result, experts claimed, a locust plague was for the first time stopped in its tracks by human intervention. But OTA argues that it is not at all clear whether it was the insecticide or just fortuitous changes in the weather that wiped out the insects.

Rather than taking credit for ending the plague, the report says that AID officials should be taking responsibility for "the mostly uncounted health and environmental costs of insecticide-based control programs; redirecting development funds to emergency efforts; and focusing on a few insects that, while highly visible, do not cause crop losses as great as some other agricultural pests". In 1986, OTA contends, donors spent $40 million and succeeded in saving only $46 million worth of crops.

A better approach, the OTA says, would accept that most locust upsurges do not result in famine. Instead of major assaults on this one enemy, what are needed in Africa are intelligent integrated pest management and monitoring systems for insects, weather and vegetation. Such an approach would deal with different pests according to their economic impact, would stress prevention over crisis management and would look for long-term development of African agriculture over emergency intervention.

The report has already provoked a strong response. Senator Patrick Leahy, chairman of the foreign operations subcommittee which funds the AID's locust control programme, described the massive use of insecticides as "absolutely unacceptable" when it costs as much to protect the crops as the crops are worth, let alone health and environmental costs.

Locust experts are united in their support for longer-term integrated pest management, but their views of the 1980s campaign make the OTA report seem too combative. Joyce Magor, head of the department of pest ecology at the UK's Natural Resources Institute, points out that a locust plague is usually stopped by a combination of events: "unusual migrations that send a proportion of the insects to a place where they cannot breed successfully, lack of rain in locust breeding areas and the coincidental arrival of locusts in countries where control is effective". In 1988, a fortuitous air current sent huge swarms of locusts on a final voyage out over the Atlantic, while some well-organized campaigns mopped up huge numbers of locusts. Neither weather nor human intervention alone can expect to take the credit for ending the plague.

Measuring the effectiveness of a campaign in terms of how much damage it averted is a "guessing game" says Magor, "because you would have to estimate where you killed them, and where they would have gone if you had not." In the 1980s plague, swarms crisscrossed northern Africa but spent most of their time in subsistence farming areas. They did not enter the regions of Morocco, for example, where crops are grown to supply out-of-season specialities to Europe and large financial losses might have been recorded. Had human intervention prevented crop loss there, OTA's sums might have added to a different conclusion.

Alun Anderson

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* *A plague of locusts — special report*, OTA–F–450 (Washington DC: US Government Printing Office, July 1990).

UK RESEARCH SPENDING

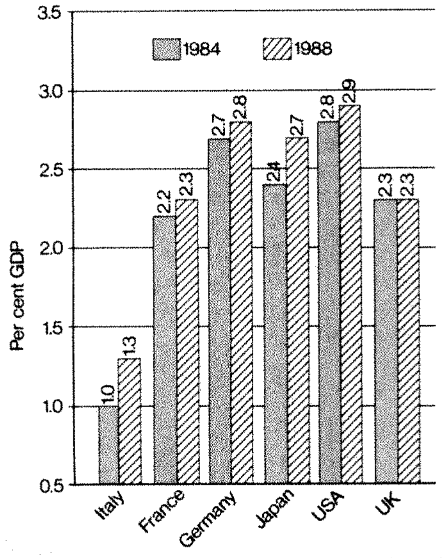
Underfunding hits patents

London

BRITISH investment in research and development is failing to match that of other nations and research output, measured by the number of patents granted in the United States, is suffering accordingly. Those are the key statistics buried in the 300-plus page *Annual Review of Government Funded Research and Development*, published by the UK Cabinet Office.

A total of £10,300 million was spent on research and development in the United Kingdom in 1988, representing an increase in real terms of 2.1 per cent from 1987. Thirty-seven per cent of funding came from government, 51 per cent from British industry and a small fraction from overseas.

But if the figures are converted to a proportion of the UK's gross domestic product (GDP), as is usual for international comparisons, British spending is seen to have stagnated at the 1985 level. In five other countries surveyed, research and development spending as a proportion of GDP is increasing (see figure), leaving Britain behind West Germany, Japan and the United States, but ahead of Italy and level with France. Even this analysis may exaggerate the British research and development effort: a recent report from the House of Lords Science and Technology Committee suggested that much of British defence research is not truly innovative and so does not conform to internationally accepted definitions of research and development (see *Nature* **345**, 5; 3 May 1990).



Gross domestic expenditure on research and development in six countries. West German and UK figures are for 1985 and 1988. Japanese figures are for 1984 and 1987.

For the first time, the Cabinet Office has included data on patents as a measure of the output of British research and development. The study looked at patents granted in the United States in order to avoid bias when making comparisons with European countries and Japan.

Britain was granted 14 per cent more US patents per capita in 1984–88 than in 1979–83. But other countries did better. West Europe was up 23 per cent on average, led by West Germany with 28 per cent. The Japanese performance eclipses that of Europe, with 79 per cent more US patents per capita granted to Japan in 1984–88 than in 1979–83.

The poor British performance is part of a long-term decline. In 1963–68 Britain was granted more US patents per capita than the West European average, with the Japanese rate less than a quarter that of the United Kingdom. But the UK rate for 1984–88, at 44.06 US patents per million population, was almost identical to the 1963–68 figure. In contrast, the West European average rose to 58.42 per million and Japan was granted 114.62 US patents per million of its population.

There are some seeds of comfort for the British scientific community. The number of academic research staff in UK universities has risen steadily over the 1980s, from 6,309 in 1983–84 to 8,413 in 1988–89, and industrial research and development spending is at a record level, at £6,900 million in 1988.

Projections for UK spending up to 1992–93 may already have been overtaken by events. A decrease in the amount of civil research and development, relative to spending in the defence sector, is forecast. But the figures are based on the government's annual statement on public expenditure made last autumn, before the political upheavals in Eastern Europe raised the possiblity of a 'peace dividend'. A predicted decline in total research and development spending in real terms may also be found incorrect when the public expenditure statement is released in the autumn.

Peter Aldhous

JAPAN'S SPACE PROGRAMME

Rocket of falling ambitions

Tokyo

JAPAN's next-generation H-II rocket will have less power than expected if and when it blasts off on its first flight in 1993.

The National Space Development Agency (NASDA) announced last week that the thrust specifications of the main engine of the rocket have had to be reduced so that the agency's engineers can meet the 1993 launch deadline.

The Japanese-made liquid fuel engine in the first stage of the H-II has been plagued with problems ever since test firings began last year. In the first test runs, the turbine blades of the liquid hydrogen pump resonated and cracked, forcing a one-year delay in the first test launch of the rocket (see *Nature* 340, 253; 1989). In subsequent tests, the engine burst into flames.

After a series of 16 test firings between March and May this year, NASDA engineers thought they had their problems solved. But earlier this month the engine again caught fire during a test run.

This time the fire had an entirely different cause. M. Miyazawa, director of NASDA's propulsion systems group, says the fire began when hydrogen leaked from a small pipe feeding the liquid fuel to the ignition system of the engine. The pipe cracked when NASDA engineers deliberately made the engine vibrate to collect data necessary for the design of a vibration suppression device which will be incorporated into the engine.

Miyazawa stresses that under normal circumstances the engine would not have caught fire. But NASDA will now redesign three pipes in the engine, including the one that broke. And, more important, NASDA engineers now realize that they cannot build a reliable engine that meets the original thrust specification of 120 tons (in vacuum) by 1993. So instead, the thrust will be reduced to 110 tons.

Naotaka Oki, director of the space development division of the Science and Technology Agency (STA) to which NASDA is affiliated, emphasizes that even with the reduced thrust the H-II will still be able to meet its original goal and place a 2-ton payload into geostationary orbit. That will be possible because the power specifications of the solid fuel booster rockets and second-stage engine were increased last year. But the rocket will not be able to lift 2.2 tons as was hoped when the new specifications were announced, Miyazawa says.

When the rocket is used for the double launch of a geostationary meteorological satellite (GMS-5) and an unmanned space platform (SFU) in 1994, extra solid fuel boosters will be added to the first stage. But this is a "one-off case" and, despite reports to the contrary in the Japanese press, extra boosters will not be required for other launches, Oki says.

The H-II's first-stage liquid fuel engine, like that in the US Space Shuttle, uses a staged combustion cycle that provides higher performance than the gas-generation cycle applied in other liquid-fuel rockets such as Ariane and Delta. But this type of engine is notoriously difficult to develop. Miyazawa admits that, even with the reduced thrust specification, meeting the 1993 launch deadline will be "tight".

David Swinbanks

NUCLEAR POWER

Reactor judged not so fast

London

ANY lingering hopes held by the UK Atomic Energy Authority (UKAEA) for a revival of Britain's flagging fast-breeder reactor programme were dashed last week by the publication of a report from an all-party House of Commons committee. The report agrees with the government's decision to end support for the prototype fast reactor at Dounreay, Scotland, from 1994 and concludes that continuing British investment in a European Fast Reactor (EFR) after construction begins in 1997 is unlikely to be worthwhile.

Fast reactor research has consumed £4,000 million (in 1988 money) of government funds since 1954. When development began, fast reactors made sense because it was thought that uranium fuel would eventually be in short supply. Fast-breeder reactors can extract 60 times more energy from a quantity of uranium than conventional thermal reactors. But there is still no shortage of uranium.

The energy select committee rejects claims from the old Central Electricity Generating Board (CEGB) that fast reactors are needed as part of Britain's strategic energy planning. Major expenditure on fast reactors can be justified only if there is "a potential economic case", and calculation of the likely future supply of uranium show that it is very unlikely that fast reactors will become economic before 2020. Further development should wait, the committee says, because of the risks of designs "becoming obsolete" before they are required commercially.

Former energy secretary Cecil Parkinson decided in 1988 that funding for the UKAEA Dounreay fast reactor should end after 1994. But Britain is still involved with France and West Germany in a plan to begin building the European Fast Reactor in 1997.

British experience with the Dounreay reactor is valuable to the project, but the United Kingdom is a junior partner financially, contributing some £10 million a year — about 15 per cent of the total. The remainder of the research and development costs are divided roughly equally between France and West Germany. But the UK contribution is expected to rise to almost £350 million in each of the two peak years of construction.

The energy committee concludes that this investment should be reviewed in 1993 and 1997, and seems at present to be unnecessary. The CEGB and UKAEA argued that British withdrawal from the EFR would wreck the collaboration, but the committee believes that France will probably continue with fast reactor development in any event. Jean Leduc, from Novatome, a French company which builds nuclear power stations, says fast reactor development is "more urgent" in France than the United Kingdom. France already relies heavily on nuclear generated electricity, and has a generation of pressurized water reactors that must be replaced between 2010 and 2020. Leduc says the EFR programme is organized to produce a commercial design by this time.

In the future, concern over global warming may change energy policy and favour power plants that produce little carbon dioxide. But the committee did not feel these considerations would affect the economic viability of fast reactors. Renewable forms of electricity generation, such as wind, wave and tidal power, are in the same situation as fast reactors — "technically proven but not [yet] commercially viable" — and other forms of nuclear electricity generation, notably pressurized water reactors, share the advanatage that they do not produce carbon dioxide but are cheaper, unless the price of uranium increases substantially.

The report says that large-scale research and development projects should be kept under "close and continuous review".

Peter Aldhous

OCEANOGRAPHY

UFC funds deep-sea centre

London

The UK Universities Funding Council (UFC) has agreed to provide £10 million for a new centre for deep-sea oceanography in Southampton. The centre will open in 1994 at an estimated cost of £43 million, mostly supplied through the Natural Environ-ment Research Council (NERC). The UFC money is to allow researchers from the University of Southampton to move to the centre and work with NERC scientists.

The centre will have an important role in climate change research, hosting the UK contribution to the World Ocean Circulation Experiment, a large project investigating the relationship between oceans and climate — a major uncertainty in current climate models. Peter Aldhous

NUCLEAR SAFETY

Kicking the habit

Munich

LIKE a person who has recently given up smoking, non-nuclear Austria is trying to persuade its neighbours that they too should give up nuclear power, for their own sake and for the sake of those around them.

Concern about the safety of some of Czechoslovakia's oldest power plants has provoked Austria's latest antinuclear crusade. Last week, Austria offered to sponsor an inspection of the two oldest reactor blocks at Bohunice, in Slovakia near the Austrian border, and to deliver electricity valued at 10 million schillings (about $830,000) a day for up to 10 days during which the plant will be shut down.

Austria chose to abandon its own nuclear programme in 1987, but construction and operation of nuclear plants in three neighbouring states — Czechoslovakia, Hungary and Yugoslavia — has rolled merrily along (see *Nature* **346**, 98; 12 July 1990). Although the Czechoslovak government ultimately agreed to allow Austrian experts to join in a safety inspecti of Bohunice by 10 August, new Czechoslovak Environment Minister Josef Vavrousek says that "there is a 99 per cent chance" that such an inspection would find the reactors safe. Austrian Environment Minister Marilies Flemming disagrees, saying that if even a fraction of the reports she has heard about Bohunice are true, it must be shut down immediately.

Flemming will travel to Hungary next week, where a spokeswoman said she would urge the Hungarians to resume construction of the huge Nagymaros hydro-eletric dam project, which the Hungarian government stopped last year in order to protect the Danube and surrounding areas. Electricity generated by the project would provide an alternative to that from nuclear power plants. After that, she will try to persuade Yugoslavia to shut the Krsko nuclear plant, which is in Slovenia, also not far from Austria.

Beyond the goodwill gesture of replacing lost power for a few days, Austria has made few offers to help Czechoslovakia finance the building of new power stations. The Austrian government estimates that it would cost $16,000 million just to bring existing Czechoslovak power stations up to Western standards. Austrian ministers met Western European leaders in Salzburg last week to try to persuade them to set up an "Energy Marshall Plan" for Eastern Europe.

A spokesman for the International Atomic Energy Agency (IAEA) in Vienna, which will carry out a limited safety inspection of its own in October, said it takes the problems with reactors of the type found in Bohunice (VVER 440-V 230) "very seriously". Such reactors are also found in Bulgaria, the Soviet Union and also in East Germany, where they were judged unsafe and ordered shut earlier this year. IAEA spokesman David Kyd said it would try to "get everyone around a table" to discuss the future of these reactors. **Steven Dickman**

WEST GERMAN RESEARCH

Greens to shut Berlin reactor

Munich

OPPONENTS of nuclear energy in Berlin are poised to shut down a research reactor and are threatening the future of an important research institution, the Hahn-Meitner Institute (HMI). HMI, named after Otto Hahn and Lise Meitner, who together with Fritz Strassmann discovered nuclear fission in Berlin in 1938–39, is the only West German national laboratory (*Grossforschungseinrichtung*) in West Berlin.

Last week, Environment Senator Michaele Schreyer of the *Alternative Liste*, the local branch of the environmentalist Green Party, refused to sign a licence for the restored research reactor at the Hahn-Meitner Institute. Ironically, she does not contend that the reactor itself presents a danger, but rather that the disposal of radioactive waste from the reactor is not and never will be assured as long as West Germany has no facilities for waste reprocessing and disposal.

The newly refurbished 10 MW research reactor was meant to be used as a neutron source for biology, medicine and chemistry as well as physics, and was completed at a cost of DM170 million (about $110 million) late last year. Since then, it has been consuming DM1.8 million a month in maintenance costs.

West German Research Minister Heinz Riesenhuber (Christian Democrat), whose ministry provides 90 per cent of the HMI budget, has threatened to withdraw support from HMI if the reactor is not licensed by 31 July. According to Hans Stiller, scientific director of HMI, if the reactor licence is denied, then there will be an exodus of researchers from the institute that could ultimately force it to close down.

Schreyer's refusal to license the reactor could be reversed when a new government is elected in West Berlin later this year, says Stiller, but such a move would certainly be challenged in court and the reactor would have to remain shut down in the meantime. Then the exodus of researchers would begin in earnest, he fears, because the wheels of justice turn "very slowly" in West Germany.

The only chance to save HMI, says Stiller, is for West Berlin Governing Mayor Walter Momper (Social Democrat) to remove from Schreyer the task of licensing the reactor. Stiller admits that this tactic would certainly bring down the fragile 18-month-old government coalition between the Social Democrats and the *Alternative Liste*.

Nevertheless, there are compelling reasons for Momper to risk the coalition in order to save HMI. Momper's Senator for Science, Barbara Riedmüller-Seel, writing in the West Berlin daily *Der Tagesspiegel*, cites the reactor's "strategic importance" for science in West Berlin. If the HMI reactor is not licensed, she argues, the city will lose out when new research institutions are created. At risk would be the electron storage ring BESSY II, another politically controversial project opposed by *Alternative Liste*.

Momper has risked the break-up of the coalition on other issues recently, in anticipation of new elections encompassing both West and East Berlin. *Alternative Liste* support is bound to wither in the reunified city, where economic concerns will dominate over environmental ones. But Momper will have to act fast if he wants to dismiss Schreyer before her decision not to license the reactor is made final. She told HMI that it would have only until 6 August to raise objections to her decision.

Although the issue has dragged on ever since the Social Democratic-*Alternative Liste* coalition took power in West Berlin, it continues to mobilize the West German research community. In July, the chairman of the West German national laboratories, Harald zur Hausen, appealed to both Riesenhuber and Momper to prevent any further delay in licensing the reactor.

Stiller and the Social Democratic majority in the West Berlin Senate contend that waste disposal for the HMI reactor is already assured. HMI last year signed a contract with the British reprocessing facility at Dounreay to reprocess its waste, return the restored fuel rods, and leave the remaining waste there for 29 years, by which time West Germany will have found a place to put its own nuclear waste.

According to Stiller, West German law requires only six years of guaranteed storage, so the current HMI arrangement exceeds the legal minimum by 23 years. But Schreyer claims that transport over such long distances is too risky. West Germany needs a plan for long-term waste disposal now, she says, or else the operation of any nuclear reactor is irresponsible.

The repercussions of Schreyer's demand may reach far beyond the future of HMI, Stiller warns. There are other *Länder* with Social Democratic governments that would be only too happy to challenge the federal government in court over the issue of waste disposal.

The new Social Democratic–Green government in Lower Saxony has announced that it plans not to continue construction at Gorleben and Konrad, which were intended to become West Germany's only sites for final storage of high- and medium-level wastes respectively. **Steven Dickman**

SCIENCE JOURNALS

Parlez-vous français?

Paris

IN an attempt to halt the decline of French as a scientific language, the Ministry of Research and Technology and the National Institute of Health and Medical Research (INSERM) are offering two FF400,000 ($72,000) prizes for the best original papers published in French in the journal, *Comptes rendus de l'Académie des Sciences*. Now that the *Annals de l'Institut Pasteur* is published in English, *Comptes Rendus* is the most respected science journal to be published in French — albeit with long abstracts in English.

A LA RECHERCHE DE COMPTES RENDUS
BIRCH

Over the past decade, French scientists have gradually abandoned native journals in favour of international English-language journals. Recently, researchers in life sciences at CNRS, the national science research centre, were more or less told by their controversial director, Claude Paoletti, that only such publications would have any weight when it came to laboratory assessments for grants — a move now rather at odds with the ministry.

The new effort to improve the image of French language publications is also an act of defiance. There is a common feeling here that papers submitted to international journals are often rejected because the researchers are not American or English.

Peter Coles

INDIA

Forest cover disappears

Bangalore

INDIA has lost 1,907 sq. km of forest cover in the past four years according to the results of a new assessment made by the Forest Survey of India using Landsat data. An earlier assessment based on satellite imagery collected between 1981 and 1983 showed that the country then had a forest cover of 642,041 sq. km. By 1985 to 1987, the period on which the new assessment is based, forest cover had declined to 640,134 sq. km. Mangrove forest cover increased slightly from 4,000 sq. km to 4,200 sq. km. According to India's Ministry of Environment, steps being taken to slow deforestation are provided by the National Forest Policy and Forest Conservation Act of 1988 and include a centrally sponsored scheme to encourage the use of fuels other than wood. Radhakrishna Rao

Geologists' Association response

SIR—Des Griffin in his letter[1] on the rumpus over the Natural History Museum's corporate plan is quite categoric. "The problem is the British government and its continued refusal to recognize the enormous importance of museums in all areas of science and education." He asks how the Geologists' Association would approach the problem — "presumably 132 years have taught them something". Quite so.

First, it is evident that if government does not recognize the importance of museum research, it is because it does not understand it. If it does not understand, it can only be because no-one has taken the trouble to explain it in language it can understand. The responsibility lies with the scientific community, not the government.

I should like, as president of the Geologists' Association, to outline the role it has played. The crisis over support for taxonomic research at the Natural History Museum is part of a much broader issue, as Griffin clearly perceives. All governments find it difficult to justify extensive financial support for long-term strategic research, data-gathering and geological surveying as well as taxonomy of animals, plants and fossils. Pure or 'blue skies' research and applied research they can grasp.

Whenever the funding of research has been discussed by bodies such as the Centre for Policy Studies, I have stressed the need for adequate support of such long-term data-collecting research.

In October last year, the Institute of Economic Affairs (IAE) Education Unit (a think-tank with the reputation of originating government economic policies) held a conference on the funding of research[2], whose purpose was to formulate a policy for consideration by government ministers. The conference was concerned not with political issues but with finding the best and most effective way of supporting research.

I spoke on the problems of funding long-term strategic research, using as examples the work of the Geological Survey and the Natural History Museum, and my case was incorporated in the final conclusions of the conference:

> "There is a whole area of research devoted to data collection. Some describe this as 'strategic research' . . . . Examples of data collecting research are the Natural History Museum with its wealth of data, and continuing collection of data, about all aspects of the animal kingdom. Such data collection, be it the examination of different species of fly or the varying composition of the atmospheric gases above the pole, may seem to have no immediate or obvious application, but such data, in all areas of science, is fundamental to research in those areas. Such data collection forms the 'dictionary' of the subject upon which other researchers can build.
>
> "This area of research risks being neglected in our funding arrangements. It has to be funded with taxpayers money, nobody else today will fund it, and needs to be funded through the research councils or specifically and directly by the government.
>
> "Even this area of research does produce an important economic return, but such is in general at one remove, it is usually not direct and not immediate."

It was at this point that the museum's corporate plan, with its deliberate decision that the scientific side should bear the brunt of the cuts, was launched on an unsuspecting world. In letters to the press, interviews and subsequent articles[3], I have drawn attention to the advice proffered to the government by its own think-tank.

As president of the Geologists' Association, I wrote to the prime minister and several of her ministers, to every member of the museum's trustees and to presidents of other learned societies. The last resulted in a joint letter to the press, requesting that the scientific redundancies be held in abeyance until the government's science-funding policy had been finally decided, on the basis of the IEA deliberations.

Meanwhile, I appealed to all members of the Geologists' Association to protest to the government and to their own Members of Parliament. The minister responsible was left in no doubt as to the deep feelings regarding the imminent demise of major areas of geology and palaeontology.

Time will tell whether or not our strategy was the correct one. I leave it to your readers to judge whether Beverly Halstead has "done little to help the museum by his recent comments".

BEVERLY HALSTEAD
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London W1V 9AG, UK.

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[1] Griffin, D. *Nature* **346**, 99; (1990).
[2] Sexton, S. (ed.) *The Funding of Research* (Institute of Economic Affairs Education Unit, London, 1990).
[3] *Daily Telegraph* 27 April; *Times* 27 April; *Guardian* 28 April; *Science* 11 May; *Guardian* 11 May; *Independent* 14 May (1990).
[4] *Independent* 25 May (1990).

SIR—Des Griffin's letter confuses me. I am not sure whether he supports Neil Chalmers, the director of the Natural History Museum, perhaps from the viewpoint of a museum director himself, or whether he is critical of the museum's corporate plan, and if so, on which grounds.

He accuses me of ignorant nonsense. But Griffin's musings (they are not logical arguments) do betray a touching political naivety, suffused with an inability to distinguish between the building called "the Natural History Museum", the existence of which is not at risk, and the functions within that building, which are not only at risk but are being destroyed at this very minute.

His senior-statesmanlike plea for joint pressure on the government presupposes that there is time. There is no time; staff morale is non-existent, staff are leaving even if their names are not on the redundancy list. The staff's request for reallocation of redundancies and jobs, published in the same issue as Griffin's letter, is inadequate.

I have no realistic solution to offer. Money is one problem, both now and for the future. The British government has not shown itself to be sympathetic to any organization that promotes public service as opposed to private profit. Like all governments, in the absence of internal motivation, the only thing it responds to is public pressure. These draft letters which Griffin disparages are part of this public pressure. The other problem is the attitude of management. Both problems need to be tackled at the same time. Considerably more public pressure is needed both from professionals and from the public for whose benefit the museum and its research staff exist.

There is a possible final solution which would perhaps meet with Griffin's approbation. It is not inconceivable that the collections and those research staff still remaining could be moved out of London and the museum in all senses handed over to Disneyland to do with as it pleases.

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Creating havoc

SIR—I was interested in the close juxtaposition in *Nature* for 5 July of the reports of the flaw in one of the Hubble Space Telescope's (HST) mirrors (page 3) and the letter from Christopher Lote (page 10) on scientists' view on the existence of God.

It is my understanding that one of the possible uses of HST was to throw light on the creation of the Universe. Perhaps God does not want us to know.

GILBERT F. RICHARDS
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Correction

The author of the first letter headed "Manned mission to Mars" in the issue of 28 June (*Nature* **345**, 760; 1990) is N. H. Horowitz. We regret having misspelled his name. □

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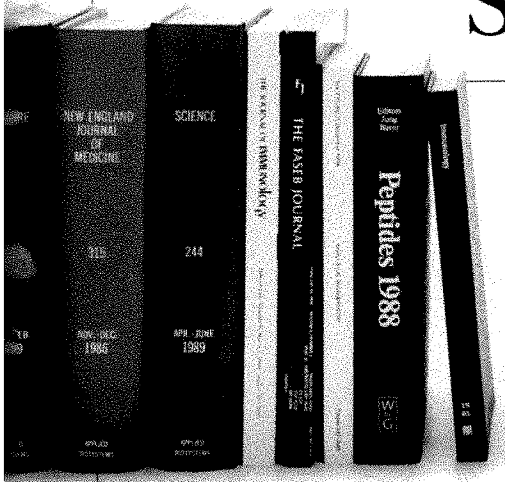

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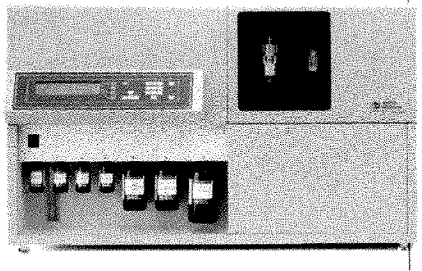

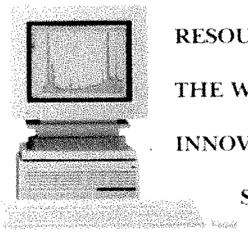

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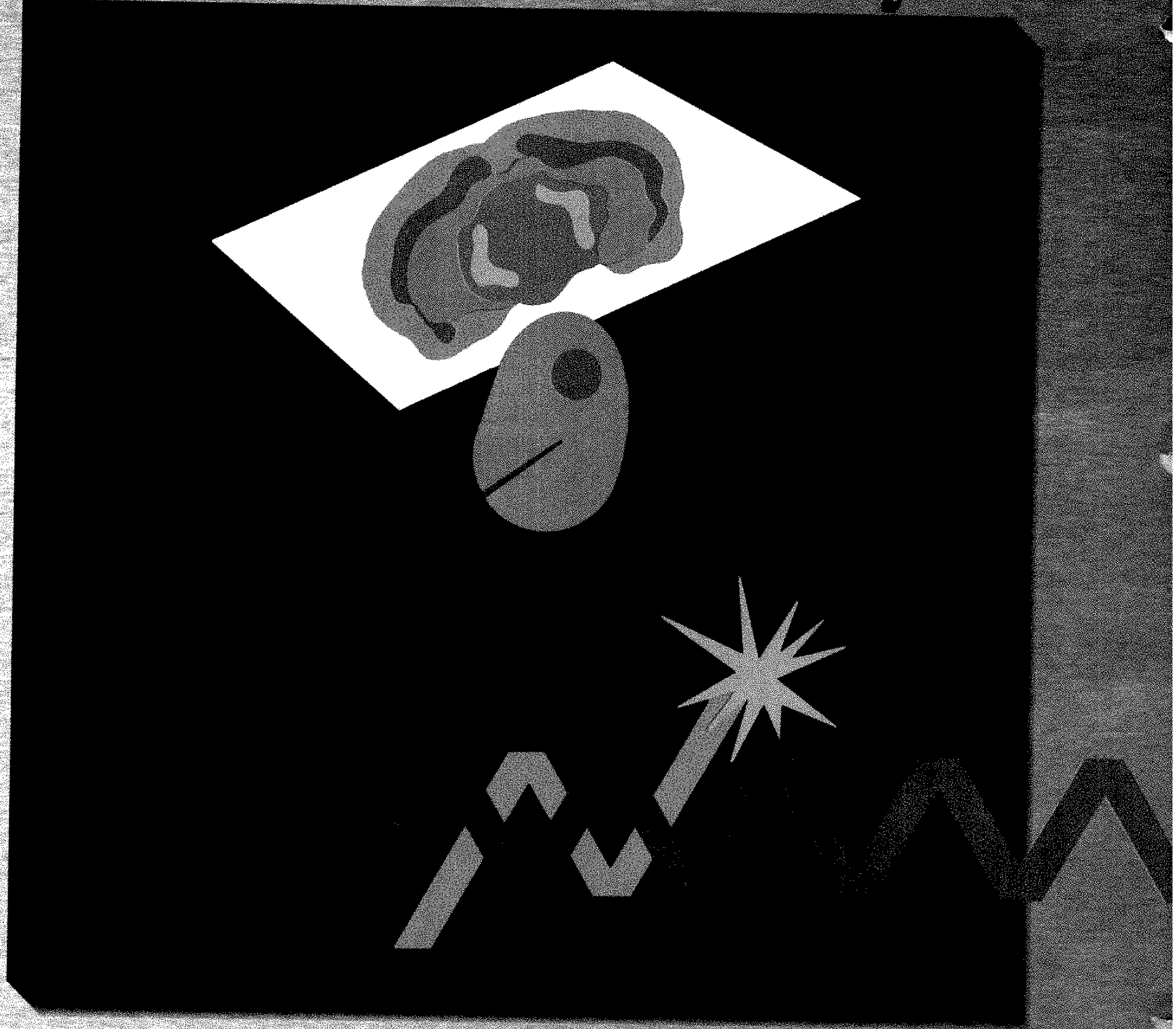

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DU PONT

Bicycling about to be explained?

A new calculation seems to have made the behaviour of bicyclists explicable — but there is a long way to go before the matter will be satisfactorily understood.

THE essential problem of the bicycle — why does a moving bicycle not tip over more often? — may at last have been solved.

That at least is the implication of a report by G. Franke, W. Suhr and F. Riess from the University of Oldenburg (*Eur. J. Phys.* **11**, 116; 1990). But one should be cautious. In the long history of mechanics, claims that the problem of the bicycle has been solved have been regularly followed by demonstrations that the claims are based on over-simple approximations that invalidate the conclusions. Ominously, even Franke and his colleagues have simplified the problem by supposing that the rider steers the bicycle only by adjustments of the position of his or her centre of gravity. It is hands-off riding. On the face of things, the solution does not accommodate what common observation demonstrates — that riders pedalling find it advantageous to make energetic movements from side to side while playing with the direction of the front wheel.

Franke and his colleagues are forgiving about previous attempts at the problem, which they acknowledge to be difficult.

They also take note of earlier demonstrations that one of the crucial determinants of stability is the distance between the axis of the steering column and the centre of the front (steerable) wheel, called the 'trail length' in the argot of the discipline. This is why the front fork of a bicycle, the device that supports the steering mechanism and the front of the bicycle frame on the front wheel, is usually bent forward towards the bottom. Their objective is to solve the problem of the moving bicycle with as few limitations as they can manage. Among other things, for example, they have allowed for wheels of unequal radius, which means that their solution should be as easily applicable to penny-farthings as to modern designs.

Much of the interest in the new attempt at the problem is the manner in which the authors have formulated it. At least superficially, it is not as complicated as might be expected.

Crudely, if a bicycle (with its rider) were a rigid body, it would have six degrees of freedom (three translation degrees of freedom and three rotations). But bicycles are not rigid: the front wheel can be steered (through a 'steering angle') while the two wheels can rotate. So there are nine degrees of freedom altogether — six intrinsic to a rigid body and three strictly internal degrees of freedom. The configuration of the bicycle at any time could in principle be defined by specifying nine variables. If the bicycle is moving, for each degree of freedom there will be a coordinate in a suitably chosen reference frame and a corresponding velocity.

But it is clear that not all possible motions correspond to stable reality. To achieve that, the two wheels, for example, must remain in contact with the ground. So the problem of the moving bicycle is that of the motion of a rigid body which is subject to what are called constraints. The simplest way of dealing with such problems is to choose a set of coordinates that will automatically ensure that the constraints are satisfied, which sometimes requires a little ingenuity.

But Franke and his colleagues make the task seem simple. Of the nine configurational degrees of freedom, for example, two are eliminated by the requirement that the wheels should be on the ground, while — at least on level ground — neither the absolute degree of rotation of the two wheels nor the absolute position and direction of the frame can be relevant to stability.

The result is that the configuration of a bicycle can be described by only two variables, taken as the steering angle and the lean angle. Of course, the dimensions of the actual bicycle enter as parameters — Franke and his colleagues use the radii of the two wheels, the trail length, the distance along the perpendicular between the centre of the rear wheel and the steering axis and the distance between the centre of the front wheel and that same perpendicular as the parameters that specify the bicycle. By similar arguments, the dynamical degrees of freedom are reduced to three — the time derivates of the steer angle and the lean angle and the scalar velocity of the bicycle (measured, for the sake of definiteness, at the rear wheel).

Turning this description of a moving bicycle into a set of equations of motion that might be solved is the more tricky part of the calculation. For practical purposes, the authors used a moving system of rectangular coordinates whose origin is the point at which the steering axis meets the ground, but whose unit vectors are along the direction of motion of the rear wheel, along the point of contact at the rear wheel with the ground to its centre and towards the hub of the rear wheel. (The last choice leads to the convenient result that the lean angle does not appear in the equations of motion.) Even so, the outcome is a vector equation (equivalent to three simultaneous scalar equations) that cannot be solved analytically, only numerically. No doubt these equations will soon be known by heart in the places at which bicycles are designed; they are a potentially endless resource for bicycle-makers.

The immediate benefits of the investigation, unfortunately, are more a spur to further investigation than a revelation in themselves. Franke and his colleagues have allowed for the bicycle rider to adjust his position laterally relative to frame. The rider's weight is supposed to be an unladylike 70 kg. One set of solutions to the equations of motions arises by supposing that a no-hands bicyclist of this size travels in a repeating circle, with a constant steering angle and a constant velocity.

Indeed, as one would expect, the greater the lean angle, the greater must be the steering angle (for a fixed velocity) or the greater the velocity (for a fixed steering angle). It is more surprising that while, above a certain critical velocity, there should be only one combination of steering angle and rider displacement that will keep the bicycle travelling in a circle at a prescribed lean angle, below that critical speed, there are two lean angles at which the same rider displacement will keep the bicycle in a circle. Those who ask whether this is a sign of incipient bifurcation to chaos will not be surprised to be answered in the affirmative.

Perhaps the most practical implication of the calculations so far carried out is that the speed at which circular motion can be stable is sensitively dependent on the moment of inertia of the wheels: the smaller the combined moment of inertia, the greater must be the velocity. Similarly, the trailing distance crucially determines the range of velocities over which motion in a circle can be stable; the greater the forward displacement of the front wheel, the greater the range of stable velocity. That, of course, is what all circus bicycle-riders know by instinct.

What all this means for real bicycle-riders is hard to tell. That the forward displacement of the rider's centre of gravity is the equivalent of lengthening the trailing distance is unsurprising.

This, no doubt, is why riders stand on the pedals when going very slowly. But what the new equations of motion mean for the behaviour of bicycle-riders remains to be determined. **John Maddox**

SOLID-STATE PHYSICS

Passing electrons one-by-one

T. A. Fulton

CURRENT in electrical devices is most often thought of as if it were the flow of a continuous, charged fluid. It is usually safe to ignore the actual discrete nature and charge ($-e$) of the individual electrons in this way because large numbers tend to be involved. Moreover, they screen each other, so that it is mostly just the average behaviour that is apparent. Recently, however, some small circuits cooled below 1 K have been devised in which current flow goes to the opposite extreme. In these 'single-electron' devices the electrons instead move one at a time in stepwise fashion, with the charge e playing a controlling role. One much sought result is to make this flow of electrons occur in lock-step with an applied radiofrequency signal, one electron per period, forming an ideal current source. A group from Chalmers and Moscow State Universities has already observed the onset of such behaviour[1], and now a team from Delft and Saclay has developed a circuit[2], called the single-electron turnstile, in which the synchronization is nearly complete, to about 1 part per 10^3. Provided that this can be extended to 1 part per 10^7 or so, such a device could become the standard of current, much as the Josephson effect is the modern standard of voltage.

Single-electron circuits employ tunnel junctions of the sort used in Josephson work. These thin-film structures consist of an ultra-thin (1–3 nm) insulating layer sandwiched between two metal electrodes. At random times electrons will pass from one electrode to the other by quantum-mechanical tunnelling through the insulator. If a bias voltage V is applied between the electrodes, these discrete, random events add up to a net current I through the junction, with a kinetic energy increment eV given to each tunnelling electron.

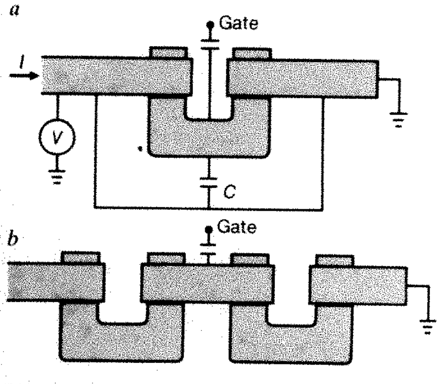

FIG. 1 *a*, Schematic of a one-electron device, comprising overlapping two Josephson junctions, metal electrodes separated by a thin layer of insulating material, with the central electrode connected to an external gate through a small capacitance (=). That electrode also has a small capacitance *C*. *b*, New turnstile device, an elaboration of the standard one-electron device. (⏚, Electrical earth.)

The essence of a single-electron device is that the current is required to pass, via tunnel junctions, through a region having low capacitance, C, to the rest of the circuit. One such configuration, of which the turnstile is an elaboration, has two closely adjacent junctions in series, with their common electrode forming the required region of low-capacitance (Fig. 1*a*). As current flows, tunnelling electrons come and go, and this region is repeatedly charged and discharged by an amount e. Each incoming electron must be able to supply an electrostatic energy of $e^2/2C$ to be allowed to enter. With junction dimensions at around 0.1 μm one can obtain C<1 fF (femtofarad) which makes $e^2/2C$ equivalent to thermal energies at around 1 K. At temperatures well below 1 K and with bias $V << e/C$ there is too little energy available to overcome this barrier and the tunnelling rate is greatly reduced. This effect, now known as the Coulomb blockade[3], was first observed many years ago in granular materials[4].

The blockade can be lifted by lowering the electrostatic potential of the low-capacitance region using capacitative coupling to a nearby gate electrode. In this case an otherwise blockaded electron borrows part of the energy needed to surmount the barrier from the gate voltage supply. This transistor-like control of the current by the gate was exploited by the Delft–Saclay group. Their turnstile device (Fig. 1*b*) had four adjacent aluminium junctions in series (total $R \sim 1$ MΩ) interconnecting three such similar low-capacitance regions ($C \approx 0.5$ fF), and was operated at about 0.03 K. With this design they achieved a sort of one-electron shift-register by applying a low static bias across the four junctions from left to right plus a radiofrequency (f) gate voltage V_G coupled to the central low-capacitance region. The electrostatic energies work out such that when the gate voltage is maximum, one electron (only) can transfer to the central region through the two left-most junctions, but not farther. As the gate voltage decreases, it becomes possible for an electron to leave the centre and escape to the right. The two conditions do not overlap, so only one electron is transferred in each cycle.

In practice all this seems to have worked well. The current–voltage characteristics with $V_G = 0$ show a Coulomb blockade region of much enhanced resistance (greater than 1 GΩ) at low bias, less than 0.2 mV. With a radiofrequency gate voltage running at a few megahertz, the I–V curve in this region develops a nice constant current step at $I = ef$, about 0.16 pA MHz^{-1}, with a shape closely matching simulations based on a semiclassical model (Fig. 2). The precision with which I/f could be measured for 4 MHz$<f<$30 MHz was limited by the difficulty in measuring the currents, a few picoamps, to within more than a few femtoamps. To give some idea of what more is needed before a standard of current can be achieved, the authors point out that for a precision of 10^8, the frequency should be less than $10^{-3}/RC$, so that sufficient time is allowed in each cycle to be confident that the random tunnelling events do take place. This probably means that the frequency should be less than 100 MHz.

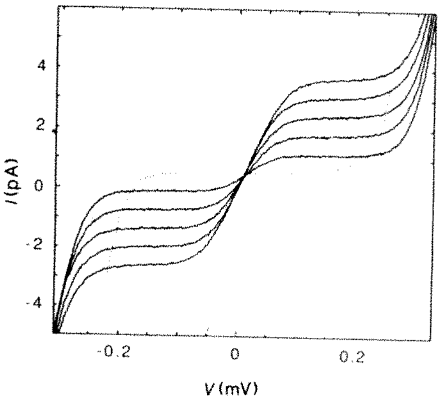

FIG. 2 Current–voltage characteristics of the new single-electron turnstile (from ref. 2). Dotted curve, no radiofrequency voltage applied. Solid curves, with applied radiofrequency voltage: current plateaus are seen at $I = ef$, where f is the applied frequency (4, 8, 12, 16 and 20 MHz) and e is the electron charge.

At the same time the number of electrons that leak through by some other path than through the turnstile mechanism must be less than $10^{-8}f$, or less than one per second. Within the semiclassical model, the only leakage is by thermally excited electrons passing over the Coulomb barrier. The authors calculate that a temperature of 15 mK would suffice to suppress these for the present device. As they note, however, it is uncertain whether a fuller quantum treatment will preserve this favourable prognosis. The Coulomb barrier itself has some spread in energy owing to its finite lifetime and the uncertainty principle, and this may cause an unavoidable leakage current. We shall have to wait and see. ☐

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PROTEIN CHEMISTRY

Pieces of the folding puzzle

Robert L. Baldwin

AFTER many years of scepticism, it need no longer be doubted that proteins go through a series of identifiable intermediate stages as they fold up to assume their native conformations. In their latest work, reported on pages 440 and 488 of this issue[1,2], Fersht and co-workers show that the folding process in the enzyme barnase follows one preferred pathway with at least one distinct intermediate state: the ghost of the 'jigsaw-puzzle' model[3], which postulates a random collection of folding intermediates, has now been laid to rest.

This new work is made possible by techniques for following the development of structure in a protein as it folds; questions can now be framed about how folding intermediates are stabilized and about the rate-limiting step in folding.

The first of the new methods[2,4,5] essentially assays the state of individual peptide NH groups at different stages of the reaction. At various times after folding has been initiated, a protein with deuterated amide groups is pulse-labelled with hydrogen. Deuterons on the amide groups exposed to solvent at the time of labelling exchange for protons that can be detected when folding is completed by two-dimensional NMR analysis; as the amide groups become involved in structure they are protected from exchange.

Fersht and colleagues use their results to support deductions about the transition state and an intermediate in the folding of barnase that are based on the rates at which mutant barnase molecules fold and unfold, and on Fersht's general scheme for analysing the nature of protein folding interactions. They find that the secondary structure of barnase is formed early on in the folding process, in agreement with the 'framework' model[6], which predicts that after an initial formation of secondary structure, pieces of the protein that have already folded form a framework on which the final folding process — fitting together the remaining side chains — can take place.

Similar results have been found for ribonuclease A (ref. 4) and cytochrome *c* (ref. 5). In the contrasting 'subdomain' model[7,8], the pieces of protein that dock to form the finished structure are already highly folded, with side chains in place and secondary structures already stabilized by tertiary interactions.

The barnase results suggest that the docking of 'preformed elements' late in folding is the rate-determining step in the folding process. Speculation is growing that the rate-limiting process involves stripping water from hydrophobic side chains as they become buried in the protein's core. Mutational studies of the transition state and intermediate are found to be particularly useful in analysing tertiary interactions during folding[1]. The barnase results suggest that most tertiary interactions are either fully formed or absent in the transition and intermediate state. Although a formal kinetic proof has not been given that the intermediates detected by exchange studies lie on the direct folding pathway, they seem to contain native-like structures consistent with hierarchical models of folding. Native-like structure is also observed in peptide models[7,8] of early folding intermediaries of trypsin inhibitor stabilized by disulphide bond formation. Because the latter are stable intermediates, their structures have been analysed by standard NMR methods. The pulse-labelling results for barnase demonstrate that its folding intermediates lie chiefly on one preferred pathway and not on multiple pathways[2].

But just as structural evidence supporting the framework model has begun to appear, protein chemists have become intrigued with molten globule intermediates[9,10]. These are stable intermediates that defy the two-state rule, according to which the folding reactions of single-domain proteins between the unfolded and the native states are completely co-operative, and stable intermediates (as opposed to kinetic intermediates) cannot be detected. Molten globule intermediates are compact and have substantial secondary structure but few if any fixed tertiary interactions; their hydrophobic side chains are not buried as in native proteins, but instead are exposed to water. They are found only in rare instances[9,10], typically at acid pH (as in the well studied example of α-lactalbumin) or when the native protein has marginal stability. Molten globule intermediates have recently been obtained for more proteins by careful adjustment of the salt concentration and choice of anion, after acid unfolding[11]. In contrast to the conceptually based framework and subdomain models of folding discussed above, the molten globule model is based simply on the observed properties of stable molten globule intermediates and on the premise[9,10] that similar forms lie on the kinetic pathways of protein folding. Fersht and co-workers find that the hydrophobic core of barnase is only partly organized in the transition state and intermediate[1], consistent with a molten globule model.

There are several good reasons for the current curiosity about molten globule intermediates. First, they violate the two-state rule: what happens to the co-operativity of folding? Second, hydrophobic side chains, buried in the native protein, are still exposed to water in molten globule intermediates[9,10]. If the burial of hydrophobic side chains is chiefly responsible for stabilizing the structures of native proteins, what stabilizes molten globule intermediates? Can hydrophobic side chains in contact with water nevertheless interact to stabilize folding intermediates? Benzene forms dimers in water, although weakly[12], and this effect provides a basis[13] for addressing the question.

Another reason for interest is the question of why the compact conformations of molten globule intermediates should be linked to the appearance of secondary structures, when there are few, if any, tertiary interactions[9,10] to stabilize the secondary structures. Unfolded staphylococcal nuclease assumes a compact conformation at low concentrations of denaturant[14] and presents a similar puzzle. It is likely that, in both cases, the compact conformation is produced by some kind of hydrophobic collapse. But why should this cause the appearance of secondary structures, which are held together by hydrogen bonds? Chan and Dill[15] suggest an answer to this question. They generate random compact conformations by folding on a lattice model: secondary structures turn out to constitute a large fraction of the resulting compact conformations. Consequently, occurrence of a hydrophobic collapse, if it happens, could be sufficient explanation for the appearance of secondary structures. Chan and Dill ask why secondary structures are so prevalent in native proteins but their result applies also to other compact conformations, such as molten globule intermediates.

Another piece of the puzzle has been added by Baum and co-workers[16], who use NMR to determine which amide protons are protected in the molten globule intermediate of guinea-pig α-lactalbumin. They find that two segments that form α helices

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in the native protein have protected protons in the molten globule intermediate (J. Baum, personal communication).

But the mystery of the molten globule intermediates now gets darker. An important motivation for studying protein folding pathways is to find the principles that relate amino-acid sequence to three-dimensional structure. Protein chemists have become used to thinking that the chief structure-determining principle is based on close packing of hydrophobic side chains in the protein core[17], but the hydrophobic side chains are still exposed to water in the molten globule. What, then, determines that the helices that form in the molten globule of α-lactalbumin are similar to those in the native protein?

As if these conundrums were not enough, Ptitsyn and co-workers[18] have now added another. They propose first that molten globule intermediates occur universally on the kinetic pathway of protein folding and then present evidence suggesting that the molten globule is formed only after secondary structure appears, which contradicts the above suggestion that secondary structures are formed because the molten globule has a compact conformation. If a hydrophobic collapse is not needed to produce secondary structures in folding intermediates, then what does stabilize them? The trail seems to be leading back towards the framework model. It is a safe bet that new developments will come rapidly. ☐

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GLACIAL CLIMATE

Ice masses on the move

Donald G. Sutherland

DURING the last glacial cycle (approximately 120,000 to 10,000 years ago) the maximum expansion of ice sheets is generally presumed to have occurred at around 18,000 years before present (BP). Glacial advances pre-dating this time are not well documented, apparently because of the destruction of evidence by that last ice advance as it over-rode the deposits of the earlier glaciations. The work of Seret *et al.*, reported on page 453 of this issue[1], is therefore of particular interest. They apparently demonstrate that at least one phase of glaciation of the Vosges mountains (at approximately 48° N) during the last glacial cycle was considerably more extensive than that which occurred at the last glacial maximum. An extensive end moraine system that marks the maximum extent of the ice is reported, and dating control on the ice advance is derived from long sequences of terrestrial and lake sediments preserved in basins outside and inside the ice limits.

The interglacial/glacial/interglacial cycle of the past 125,000 years is important in elucidating the mechanisms controlling the Earth's climate. As it is the most recent such cycle, the relatively well preserved and geographically widespread deposits dating from it allow detailed reconstruction of particular events. The period offers examples of large, rapid changes in climate as at the end of the last cycle when global temperatures may have increased by around 5 °C over a few centuries (or less). It is also possible to recreate the palaeogeography and palaeoclimate of certain periods characterized by climates markedly different from the present climate and to use these reconstructions to test computer models of global circulation.

The distribution of glacial ice is of great importance for palaeoclimatic modelling. Ice has a crucial effect in reflecting solar radiation: the largest ice sheets were of sufficient size to modify the general circulation. And individual ice masses also had direct effects on local climate, modifying, for example, precipitation patterns in their immediate vicinity. But although the general trends in climate during the last glacial cycle have been indicated by analyses of deep-sea sediments or ice cores from the Greenland and Antarctic ice sheets, these continuous records are unable, in other than a rather general way, to indicate the geographical expression of the environmental changes they record. Thus, for example, although it may be possible to infer that a certain volume of ice existed during a certain period, little can be deduced as to where that ice had accumulated. Similarly, rapid changes in temperature, whether positive or negative, can be inferred from the continuous records, but this tells little of the environmental response to these changes. It is necessary, therefore, to turn to the continental record to discover the evidence relevant for palaeogeographical reconstructions.

In their study[1], Seret *et al.* describe a series of terminal moraines formed by an ice mass emanating from the Vosges mountains in France. They establish that these moraines are more than approximately 30,000 years old, from the sequence of sediments in the Cusenier basin which lies within the outermost limits of the ice advance. In addition, they use the long sequence of terrestrial environmental change interpreted from the sediments preserved in the neighbouring Grand Pile peat bog to suggest that the most probable period for the glaciation was between 30,000 and 50,000 BP. They do not rule out the possibility of earlier glaciation, however, nor, as the moraines marking the limit of the ice advance are multiple, the possibility that there was more than one period of glaciation.

Their favoured timing of the glaciation falls within oxygen-isotope stage 3, a period some would consider relatively mild, although one in which the deep-sea record indicates there to have been substantial global ice volume. As noted by Seret *et al.*, the southern margin of the Laurentide ice sheet was apparently well advanced during this period[2] and comparison may also be made with the western Norwegian sequence[3] where glaciation of the coast during the Skjonhelleren Stadial (between approximately 60,000 and 30,000 BP) has been inferred.

The dynamics of an ice mass is a question of balance between accumulation and ablation, the former generally being aided by increased solid precipitation and the latter largely being controlled by temperature. As the precise balance between these two major variables for a particular area may differ somewhat from one cold period to another then the dimensions of any consequent glacial build-up need not be similar in any two such periods. Seret *et al.* use evidence from minerals and pollen from the Grand Pile bog to address this problem and suggest that the principal local control on ice expansion and contraction in the Vosges was precipitation rather than temperature.

Thus they envisage the ice building up during a period of cool but not extremely cold climate, a period characterized by high snowfall and with the ice in retreat as much in response to a decrease in snowfall as to an increase in temperature. Although they do not directly state it, there is the further possible implication that a different combination of precipitation and temperature occurred during the build-up of the ice immediately before about 18,000 BP. This could explain the smaller ice mass that developed in the Vosges at the later time.

This work of Seret *et al.* highlights a current lack of knowledge about major ice masses during the last glacial cycle. With the broad temperature trends during this period already relatively well known, more work along these lines could yield a better idea of the contemporaneous changes in precipitation patterns. With that, we would have a near-complete picture of the glacial climate. ☐

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PRIMATE EVOLUTION

African dawn for primates

Philip D. Gingerich

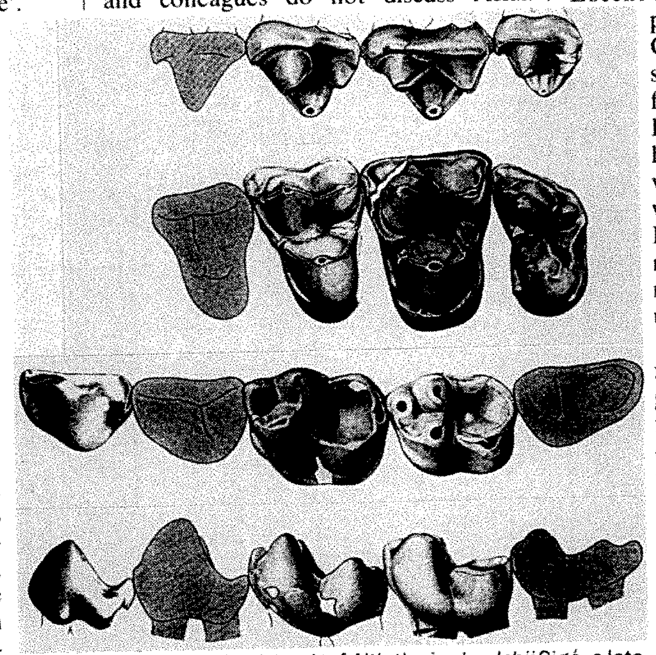
THE fossil record of primates is one of the most intensively studied of all major mammalian groups. But until recently, fossils of the most ancient true primates were absent from Africa, where primates are a diverse faunal component today. This gap has now been filled with the discovery of the first true primate from the Palaeocene of Africa, by Sigé *et al.* at the University of Montpellier in France[1].

This new find, *Altiatlasius koulchii* Sigé, comes from late Palaeocene sediments at Adrar Mgorn, in the eastern Quarzazate Basin of Morocco, at the foot of the High Atlas mountains, a locality discovered in 1977 (ref. 2). Fossils occur in a hard calcareous matrix deposited in a nearshore marine setting. These are fragile and extracted with difficulty in acid. Shark teeth indicate that the age is Thanetian (late Palaeocene, about 60 million years ago)[3]. Twenty-three mammalian species are represented in the Adrar Mgorn fauna[4,5], some by beautiful dentaries and maxillae, but most by isolated teeth. Small mammals predominate, especially insectivorous species. All are eutherians, including genera familiar in the Palaeocene of Europe or North America (*Aboletylestes*, *Cimolestes* and *Palaeoryctes*) showing that communication with northern continents was possible at times.

The new primate is represented by ten isolated cheek teeth (see figure). One dentary fragment of a juvenile preserves a single erupting molar, but no two teeth were found in association. The upper molars are trapezoidal at the base with a broadly basined trigon. The lower molar trigonids are low and talonids too are broadly basined. Tooth size indicates that *Altiatlasius* was comparable in body size with the mouse lemur *Microcebus murinus* or Demidoff's galago *Galago demidovii*. In life, *Altiatlasius koulchii* probably weighed no more than 50–100 grams.

Sigé and colleagues assign *Altiatlasius* to the family Omomyidae and regard it as the oldest haplorhine and the oldest true primate. They note close resemblances with younger, possibly more derived forms such as *Omomys* and *Chumashius* from the Eocene of North America[6] and *Kohatius* from the Eocene of southern Asia[7,8].

The single known premolar is unusual and does indeed suggest an affinity with *Kohatius*. Sigé and colleagues also compare *Altiatlasius* favourably with the contemporary plesiadapiforms *Berruvius* from the Palaeocene of Europe and *Micromomys* from the Palaeocene of North America, and to a lesser degree with the adapid *Donrussellia* from the early Eocene of Europe and the adapid or catarrhine *Oligopithecus* from the Oligocene of Africa. Bulbous cusps on lower molars remind me a little of *Cantius*. Sigé and colleagues do not discuss Asian *Altanius*, but *Altiatlasius*, like *Altanius*, is sufficiently primitive that it does not fit clearly into any single familial grouping[9]. Sigé and colleagues are probably right that *Altiatlasius* is an omomyid and the oldest true primate, but isolated teeth are difficult to interpret and more complete specimens with anterior teeth will be required to remove some lingering doubt.



Left upper and lower cheek teeth of *Altiatlasius koulchii* Sigé, a late Palaeocene primate from Adrar Mgorn in Morocco. Upper molars (top) are shown in lateral and occlusal view. Lower molars (bottom) are shown in occlusal and lateral view. The largest upper molar is the holotype, and it measures 1.75 mm in length and 2.45 mm in width. No teeth were found in association, so the position of each in the tooth row is necessary conjectural. Drawings by Ariane Beaux and Christian Pondeville.

The African origin of primates is an old idea[10], but fossil evidence to support it has emerged only in recent years. The discovery in 1975 of *Azibius* in Eocene sediments in Algeria[11] — the most ancient primate then known from Africa — helped convince me that primates originated in Africa[12], although others preferred a centre of origin in central[13,14] or southern[15] Asia. Since then, further discoveries in Algeria[16] and Egypt[17] have added weight to the idea of an African origin. Now we have *Altiatlasius* from the Palaeocene of Morocco. Another new form, from the Eocene of Tunisia, is under study at Montpellier by Hartenberger and Godinot. Taken together, these specimens provide strong support for an African origin of primates. Diversification in Africa in the late Palaeocene followed by northward dispersal when climates warmed globally across the Palaeocene/Eocene boundary[18] may explain why true primates are not found on northern continents until the early Eocene.

Sigé and colleagues conclude by ranking *Altiatlasius* as the sister group of Anthropoidea (Simiiformes), and suggest that it indicates, first, that anthropoid primates differentiated during the Palaeocene, and second, that platyrrhine anthropoids (New World monkeys) rafted the Atlantic Ocean in the Palaeocene when Africa and South America were closer together. Some doubt remains that *Altiatlasius* is a true primate (after all, its teeth compare well with some plesiadapiforms), and it is certainly not an anthropoid. If *Altiatlasius* is not an anthropoid, then it does not prove anthropoids existed, let alone differentiated, in the Palaeocene: and crossing the South Atlantic in the Palaeocene would have been a big stretch when *Altiatlasius* seemingly could not cross the Tethys Ocean to Europe. ☐

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DEVELOPMENT

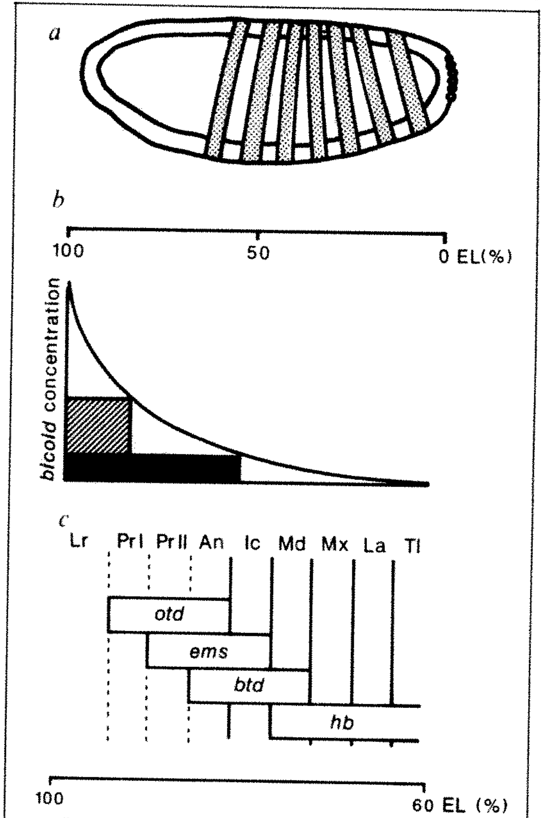
The X, Y, Z of head development

P. W. Ingham

MOLECULES that can trigger a series of biochemical switches are much sought after by developmental biologists, so the cloning and characterization of the *Drosophila* gene *bicoid* has generated considerable interest and excitement over the past two years. Yet despite being hailed as a paradigm *par excellence* of such a biological morphogen[1], a good deal of mystery has continued to surround the workings of the *bicoid*-encoded homeodomain protein in the early embryo. Although displaying the proverbial graded distribution[2], the capacity of the *bicoid* protein to evoke a series of threshold responses has been difficult to prove other than in principle, because its targets have for the most part remained obscure. Several recent studies[3-5], two of which are reported on pages 482 and 485 of this issue[4,5], have now identified such target genes, opening the way to a detailed analysis of the molecular basis of threshold responses.

A chief obstacle to the full understanding of *bicoid* (*bcd*) function has been the dearth of information about genes controlling development of the head, the part of the embryo requiring the highest levels of *bcd* activity. Posterior to the cephalic fold — a deep invagination of cells which separates the presumptive head from the rest of the embryo during early gastrulation — the appearance of primordial segments can be readily visualized through the striped expression patterns of pair-rule genes such as *fushi tarazu* (see figure). These genes are pivotal in the early development of the embryo, effecting the transition from aperiodic regional differences, generated by the selective expression of gap genes (such as *hunchback*, *Krüppel* and *knirps*), to the repeating patterns that underlie its segmental organization[6]. Anterior to the cephalic fold, by contrast, pair-rule genes seem to have little if any function and their expression is correspondingly sporadic (*hairy*, *runt*, *paired*) or, in the cases of *even-skipped* and *fushi tarazu*, nonexistent.

Whereas pair-rule genes have been useful in monitoring the consequences of altered levels of *bcd* activity[2,7], the effects of such alterations on their expression are in fact somewhat limited: in embryos lacking all *bcd* activity, the two anteriormost stripes disappear, but decreasing or increasing levels of *bcd* protein simply decrease or increase the void in pair-rule expression at the anterior end of the embryo. What has remained mysterious is the identity of the genes that fill this void and specify the organization of the head.



a, Expression of *fushi tarazu* in the blastoderm embryo is restricted to the prospective 'trunk' region of the embryo and absent from the prospective head (left) where *bcd* levels are highest (see *b*). *b*, Graded distribution of *bcd* protein and the expression domains of *hunchback* (shaded) and the hypothetical *gene X* (hatched) (adapted from ref. 2). *c*, Overlapping functional domains of *otd*, *ems*, *btd* and *hb* in the head segments as deduced from mutant analysis (adapted from ref. 5).

In the absence of putative head-specifying genes, the search for *bcd* targets was focused initially on the gap gene *hunchback* (*hb*). In common with other gap genes, *hb* controls the generation of particular pair-rule stripes, specifically the two most anterior which disappear in *bcd* mutant embryos. Zygotic expression of *hb* extends, like *bcd* protein, from the anterior pole to the middle of the embryo (100–50 per cent egg length (EL)) and is absolutely dependent upon *bcd* activity[8]. In several papers published last year, Driever, Nüsslein-Volhard and colleagues[9-11], and Macdonald, Struhl and Struhl[12], demonstrated that the *bcd* protein acts directly on regulatory sequences upstream of the *hb* promoter to activate *hb* expression. Although this finding was gratifying inasmuch as it demonstrated the transcription factor-like character of the *bcd* protein, it shed little further light on the significance of the graded distribution of the protein.

But what was more exciting was the discovery that different parts of the regulatory sequences can elicit different responses to the *bcd* gradient. DNA footprinting analysis revealed two distinct types of binding sites upstream of the *hb* promoter, one of which binds *bcd* protein with high, the other with low affinity. Whereas the high-affinity sites alone are sufficient to drive expression of a reporter gene throughout most of the *bcd* concentration range in a manner similar to the endogenous *hb* gene, the apparently redundant lower affinity sites, can, when isolated, direct expression in a much narrower domain, restricted to the anteriormost quarter of the embryo where *bcd* levels are highest. Because the absence of *bcd* results in more defects in anterior development than those caused by *hb* mutations, it had already been recognized that additional genes should be under the control of *bcd*: but here was the first indication of where such a gene — termed *gene X* by Nüsslein-Volhard and colleagues — might be expressed and how such an expression domain might be defined (see figure).

In December 1989, Dalton, Chadwick and McGinnis[3] described the cloning of a possible candidate for *gene X*, the *empty spiracles* (*ems*) gene. At first sight, this seems an unlikely match because the name *empty spiracles* refers to a defect in the tracheal system originating at the posterior end of the embryo. But more detailed analysis of *ems* mutants suggested an additional role for the gene in the differentiation of specific head structures, an inference made more compelling by analysis of the spatial regulation of *ems* expression. The gene was cloned by virtue of the fact that it encodes a protein with a homeodomain, distantly related to that encoded by *even-skipped*. Using antibodies specific for the *ems* protein, Dalton *et al.* showed that its expression is initiated in the blastoderm embryo in a wedge-shaped band of cells between 70 and 88 per cent EL, overlapping the *gene X* domain. Moreover, the position of this band depends critically on *bcd* activity, shifting respectively anteriorly or posteriorly with decreasing or increasing doses of the gene.

An even closer fit to the artefactual *gene X* expression pattern is provided by the distribution of transcripts of the *orthodenticle* (*otd*) gene, described by Finkelstein and Perrimon on page 485 of this issue. Like *ems*, *otd* encodes a homeodomain protein[13], in this case exhibiting a similarity with the homeodomain of the *bcd* protein itself. Expression of *otd* is also restricted initially to the anterior part of the embryo, in a uniform band of cells normally located between 70 and 90 per cent EL, the posterior limit of which is sensitive to decreased or increased *bcd* dosage. A clear implication of these studies is that both *otd* and *ems* are activated in spatially restricted domains in the blastoderm embryo in direct response to levels of *bcd* protein. Whether this response is mediated via the same low-affinity binding sequences as those located upstream of *hb* will doubtless be revealed soon.

The *otd* mutation was originally characterized as showing defective head involution[14]. This rather gross phenotypic description reflects the difficulties in analysing the final intricate pattern of the head segments in the mature larva — largely due to the complex topological rearrangements occurring in the head during embryogenesis — which have bedevilled the study of this part of the animal. To circumvent this problem, Finkelstein and Perrimon used the products of the *wingless* and *engrailed* genes as molecular markers for head segments in early embryos, and they were thus able to identify unequivocally those segments missing in *otd* mutants.

Using a similar approach, Cohen and Jürgens[3] have extended this analysis not only to *empty spiracles* but also to mutations of *buttonhead* (*btd*), a third gene that affects head development. Their findings reveal overlapping requirements for the activities of all three genes in the specification of each of the head segments anterior to the cephalic furrow (see figure). Because the functional domains of each gene appear to be out of register by one segment, Cohen and Jürgens propose that they act in a combinatorial fashion to specify the boundaries of each of the head segments, probably by directly regulating the expression of *wingless* and *engrailed*. They may also determine the identities of each segment by regulating the expression of head-specific homeotic genes such as *labial* and *Deformed*[15,16]. In this way, these head-specific 'gap' genes would subsume the functions normally executed by gap and pair-rule genes in the more posterior segments of the embryo.

Although this interpretation is persuasive, the precise relationship between function and expression of the three genes remains unclear: although *ems* is evidently required more posteriorly than *otd*, for instance, the domains of expression of both genes appear to be essentially coextensive. But if shown to be correct, there is an intriguing irony in all of this. Recent studies of the hindbrain in chicks and mice[17-19] suggest a close analogy between metamerism in invertebrates and an underlying segmental organization of the vertebrate nervous system. This analogy has been much strengthened by the pair-rule-like expression of the mouse *Krox 20* gene — yet it would now seem that the comparable region of the fly embryo may be segmented by a quite distinct mechanism, independent of pair-rule genes. Had the analysis of *Drosophila* development begun with the *bcd* system, the specification of head segments by overlapping domains of homeobox proteins might well have been established as the archetypal gene hierarchy: what then might have been made of zinc-finger protein stripes in alternate rhombomeres? □

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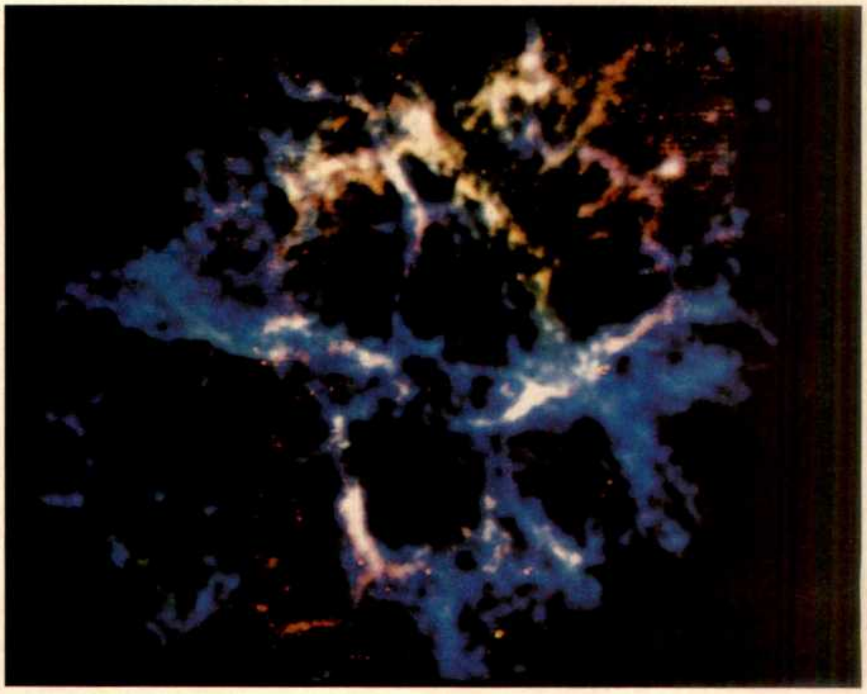
Fine filaments of the Crab Nebula



ILLUMINATED by a powerful pulsar at its heart, the Crab Nebula has continued to fascinate astronomers since it was discovered by Lord Rosse in 1844. What we see are the gaseous ejecta from a massive supernova that Chinese observers could see even during daytime 940 years ago. The nebula is now 4 light years across and is expanding at a rate of 1,400 km s^{-1}. The filaments traced out by the ejecta are the result of turbulence and shockwaves. In making this new three-colour overlay figure (*Astrophys. J.* 357, 539–547; 1990), Jeff Hester and colleagues intend to understand better the chemical composition of the nebula: red indicates iron (recorded using new infrared imaging), green, hydrogen and nitrogen, and blue, oxygen. In fact the varying colouring of the filaments indicates on the whole not varying composition, but rather differing conditions, although an excess of hydrogen swept up from the interstellar medium is apparent above the middle of the figure. One aim of the study is to tackle the iron–nickel problem in astronomy. The best measure we have of the cosmic abundances of the elements comes from studies of the composition of the Sun and meteorites formed billions of years ago from gas clouds comprising hydrogen and helium and enriched with heavier elements by supernova remnants similar to the Crab Nebula. Yet these measurements indicate that there is 10 times more iron than nickel, whereas their apparent abundances in supernova remnants and in star-forming clouds are equal. The authors conclude in the new study that the problem must arise through difficulties in the interpretation of the atomic data, and does not reflect a real variation in astrophysical abundancies. □

PLANETARY EVOLUTION

The Hyperion hypothesis

William B. McKinnon

ORBITING just beyond Titan in the Saturn system is Hyperion, a 350 × 240 × 200 km icy fragment of a world (Fig. 1). Its curious shape strongly suggests that it is the remnant of a catastrophic breakup. Indeed, it is generally thought that during the first few 100 million years of the evolution of the Solar System, all of the inner satellites of Saturn, Uranus and Neptune were repeatedly shattered by collisions and then reassembled. What sets Hyperion apart from many of these ostensibly rebuilt moons is its shape — apparently, its fragments were not able to re-accrete into the relatively spherical form usually expected for satellites of its size. This suggests that Hyperion is itself only a fragment of a previously more massive satellite and that the other pieces (or most of them) did not return home. Exactly where Hyperion's fragments might have gone, or more specifically, which other satellites might have captured them is the subject of a recent paper by Farinella *et al.*[1].

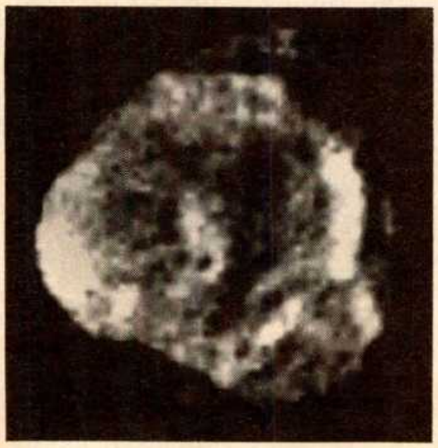


FIG. 1 Saturn's Hyperion: remnant of a protosatellite and father to a crater population?

Actually, the matters addressed in this paper are broader than that. The central issue is the origin of crater populations, and the overall subject is nothing less than the bombardment history of the Solar System. This history is the result of the complex interaction of impactor sources and dynamic delivery mechanisms, and their evolution over time. Most impacts, however, occurred very early in the Solar System's lifetime, and are attributable to remnant populations from the accretionary processes that originally built the planets and their satellites. Thus, the heavily cratered surfaces record the last stages of accretion of the Solar System. If properly deciphered, this record would help us to understand the construction of our Solar System — and others, if they exist.

Hyperion's fragments enter into this through the interpretation of crater counts, or size-frequency distribution curves. Some researchers regard the heavily cratered terrains on most planetary and satellite surfaces as having essentially the same distributions[2]. Others interpret the differences in form between distributions to be significant. Conclusions concerning the impactor populations are just as varied. Some hold that nearly all cratering in the Solar System was been caused by comets, both ancient and modern[3]. Others advocate that the different forms of crater distribution curves for different bodies must be explained by physically different impactor populations. In particular, Strom, one of the co-authors of the new paper[1], argues that the jovian, saturnian and uranian satellites have been bombarded by different populations, and thus rejects the idea that comets, the plausible generic impactors of the outer Solar System, were the cause. He holds that each satellite system was bombarded by its own, unique impactor population that originated from orbits around the parent planet[4].

Saturn's icy satellites, in addition, have been held to record two distinct populations. One population (number I) is found on the ancient, heavily cratered regions of Tethys, Dione and Rhea, and also dominates inner Mimas; the other (number II) is also found on most heavily cratered surfaces, and is superposed on the younger, resurfaced areas of Enceladus, Tethys and Dione. The younger population II has a relative excess of small craters less than about 20 km across, and is thus recognized on heavily cratered Rhea, for example, by a bump in the crater size–frequency distribution (Fig. 2). The original hypothesis for the origin of population II craters was secondary cratering: the debris scattered by population I impacts would create subsidiary impressions[5]. This idea was expanded to include the distributed effects of the collisional breakup of small satellites[6], and Strom focused this hypothesis on Hyperion[7]. More importantly, recognition of a system-wide breakup event or events offers the possibility of establishing internal clocks for the geological evolution of Saturn's satellites.

Farinella *et al.*[1] track the fate of Hyperion's fragments by realizing that the agent of their misfortune is its neighbour, the (relatively) massive moon, Titan. Had Titan not been where it is, much of the debris from an impact on Hyperion would have rapidly re-accreted onto the original moon, albeit in a reordered form. But as it is, the orbital periods of Hyperion and Titan have an exact mathematical relation of 4:3 (with Hyperion's the longer), and over repeated orbits this resonance can cause the orbits of fragments to evolve chaotically, with drastic consequences. Even those fragments with velocities that would otherwise have left them in a belt relatively close to Hyperion would find themselves pulled into orbits that crossed Titan's[1]. This was the likely cause of Hyperion's inability to re-accrete.

Farinella *et al.* consider Titan's

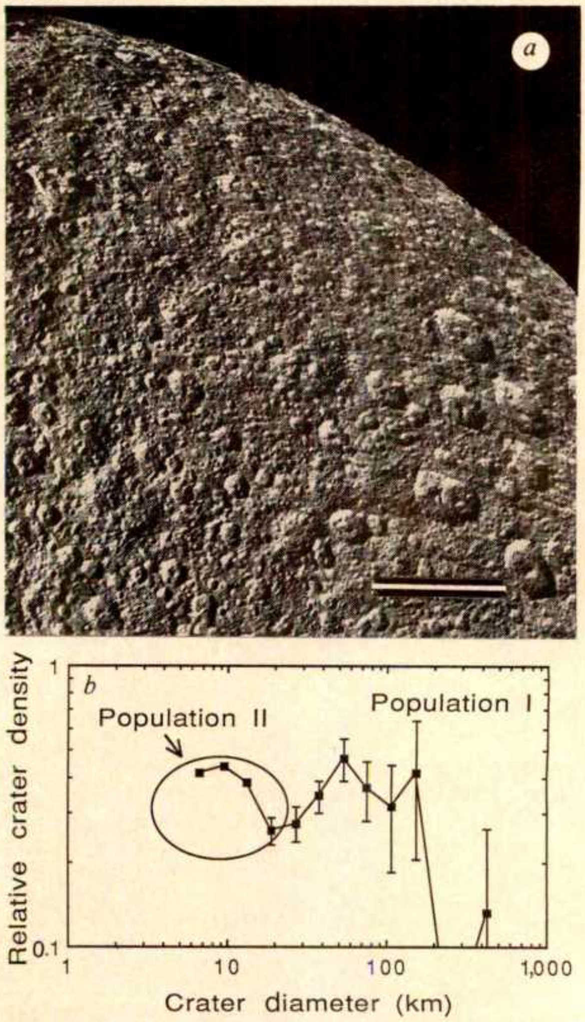


FIG. 2 *a*, A portion of the north polar region of Rhea, as seen by Voyager 2. Population II manifests as the peppering of small craters (scale bar, 100 km). *b*, Statistically, population II shows up as a bump in the relative incremental size–frequency distribution[4], whereby the areal density of craters per diameter (*D*) bin is normalized (divided) by D^{-3}.

influence on two classes of Hyperion's fragments, those that chaotically evolved so that they barely crossed Titan's orbit and those that were placed directly into Titan-crossing orbits. This latter class arises from the uncertainty in the velocity distribution of ejecta from breakup events. Outcomes are determined using Öpik's statistical theory of orbital diffusion[8]. The robust inferences are first that Titan swept up the lion's share of any fragment swarm; and second that of those fragments that did not rain down on Titan (up to around 10^{-4} of the total), Rhea, the next satellite in, got the majority. The amount of disrupted mass reaching each successive inner satellite decreases sharply. The amount of material reaching inner Mimas, which is dominated by population II craters, was negligible.

What this means is that the Hyperion hypothesis fails: Hyperion was not the source of system-wide planetocentric cratering events. Furthermore, Titan, by virtue of its relatively great gravitational cross-section, acts as a dynamic shield in the Saturn system. Any planetocentric impactor population lurking beyond Iapetus, Saturn's outer moon, which is the only plausible place to store long-lived planetocentric impactors[9], would have had relatively little effect on the satellite system inside Rhea. A non-negligible fraction of Rhea's population II craters may have been caused by Hyperion's fragments, however. To account for all of Rhea's population II craters requires, for plausible mass distributions of the fragments, a total ejected mass of the order of Hyperion's present mass, if indeed about 1 in 10^4 fragments hit Rhea[1]. Because the sphere that just encloses Hyperion's present figure has three times its present volume, this mass requirement is not implausible.

If Hyperion (or some hypothetical trans-Iapetus population) was not the cause of the population II craters on Mimas, Enceladus, Tethys and Dione, how were these craters formed? It would seem that local sources are still the most likely, that is, ejecta from giant craters or basins, such as exist on Mimas, Tethys and Rhea, and debris from the collisional breakup of small satellites such as the irregularly shaped Trojan companions to Dione and Tethys. That Rhea's population II craters could be caused by ejecta from its large basin Tirawa is not far-fetched, because the efficiency of Rhea's accretion of Hyperion debris of around 10^{-4} is actually a rough upper limit that depends on favourable geometrical or dynamic circumstances. As to the ultimate origin of these large craters and small satellite breakups, they were most likely caused by heliocentric impactors, probably comets or comet-like bodies[10].

Although the work of Farinella *et al.* elucidates only one aspect of the bombardment history of the Solar System, it illustrates the complexity of the problem very neatly. Much further work can be done, even for the Saturn system, using the full numerical armamentarium of celestial mechanics. As with much in chaotic dynamics, we should expect surprises, even startling ones. ☐

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EVOLUTION

The language of the genes

N.H. Barton and J.S. Jones

GENETICS is a language, a set of instructions passed from one generation to the next. Genes and languages each possess a history and have changed as their carriers succeeded each other and moved over the world. The history of language — its evolution — can be traced from the similarities and differences of living tongues and their literary fossils. In the same way, shared genes can be used to trace ancient patterns of relatedness. Two new papers[1,2] have uncovered some remarkable parallels between the geography of genes and of language which suggest that they have a common evolutionary history.

There are obvious analogies between linguistic and genetic evolution. Each depends on the origin of new coding elements by mutation, on random or directed change in their frequency, and on their spread by diffusion or migration. The idea of an evolutionary clock was first proposed in the nineteenth century by a linguist — appropriately enough, some might claim, Grimm of fairy-tale fame — who suggested that the differences between living languages could be used to trace their divergence. Both the phonetic and the molecular clock keep bad time if, as is true for certain proteins, bird songs and human speech patterns, some elements reproduce more effectively than others[3-5].

The geographical link between genes and language emerges from studies of many thousands of Europeans scored for cell-surface antigens and polymorphic enzymes. New statistical tests[1] show that Europeans with the same language are on average more genetically similar than are those speaking different tongues. Geographical trends in the frequencies of 63 alleles reveal 33 sharp boundaries which separate genetically distinct populations[2]: 26 of these coincide with differences in language and five are boundaries between dialects. Some populations — such as the Basques — are linguistically and genetically quite different from others.

In spite of these linguistic effects, most genetic variation is due to proximity, and neighbouring populations cluster without much regard to linguistic relatedness[6]. The fit between genes and language is subtle and can be seen only after sophisticated statistical analysis. The general pattern is of localized random fluctuations, probably produced by a balance between random drift and the diffusion of genes from place to place. Only on the larger scale are past movements of whole populations manifest as boundaries between languages.

Mass Movement

Local diffusion and mass migration — whether expansion from within an existing range, or by invasion from outside — are extremes in the complex picture of human gene flow. From the Old Testament to *Mein Kampf* history has seen mass movement as the key to the peoples of the world. By contrast, population geneticists have concentrated on within-population processes such as genetic drift and the diffusion of genes. These may be enough to explain the local differentiation of tribal and agricultural peoples, but they cannot be the whole story. Because genes diffuse through settled human populations very slowly — with a standard deviation of perhaps 1 km in a year[7] — it would take around 10,000 years for a new mutation to spread over 100 km. Mass movement must have helped to determine the population structure of modern humans. Concordance between unrelated characters usually indicates a meeting of differentiated populations, as opposed to differentiation *in situ*[8]. The European genetic boundaries and their coincidence with language probably reflect such secondary contacts.

The population genetics of bulk migration has scarcely been explored even though, over large scales, it must be more important than gradual diffusion. Phylogenies based on mitochondrial DNA in various creatures suggest that it is widespread[9]. In many species, plots of genetic against geographic distance show that widely separated populations are much more similar than expected by diffusion alone[10]. There is a similar pattern in the relationship between language and geography in Polynesia[11].

Trends in gene frequency from southeast to northwest Europe may reflect demic diffusion[12], a process intermediate between local diffusion and mass migration. As early farming communities expanded and spread west, they interbred with local hunter–gatherers. Genes in the east are typical of the newcomers, whereas those in the west retain an admixture from the original inhabitants. Most European languages — excluding Basque and a few others — belong to the Indo-European family which may itself have its roots in the origins of agriculture in the Fertile Crescent[13].

Horizontal transmission

Unlike genes, language can spread horizontally. In some places, this has destroyed the relationship between genetic heritage and speech. In the Pacific, for example, there is a sharp genetic change near Fiji in spite of a smooth gradient in language[14]. Bird-song dialects rarely last for more than 20 generations, and are transmitted by learning, with no genetic differenes between populations with distinct dialects[15].

Shared patterns of human genes and language around the world suggest, however, that such horizontal transmission was not widespread in the sparse human populations of earlier times, as the parallels between the evolution of genes and language reach back into prehistory. A phylogeny of 42 human populations based on 120 gene markers fits well with a world tree of language superfamilies[16]. For example, there is an ancient genetic split between African and non-African populations which accords with the distinctiveness and diversity of sub-Saharan languages. The origin of language could even have been the catalyst for the spread of modern humans across the globe.

A language is a group of mutually intelligible dialects. This definition is close to that of a biological species: a group of actually or potentially interbreeding populations. Like species, languages are more-or-less closed evolutionary units which cannot easily be penetrated. As it is hard to measure gene flow or mutual intelligibility, taxonomists and linguists are forced to use morphological criteria when defining the units they work with.

A problem common to linguistics and biology is just why such morphological units exist: why should inherited information be divided into distinct clusters? Perhaps clustering is an inevitable consequence of evolution. Anything which — like species and languages — evolves by descent with modification automatically produces discrete taxa[17]. This may be the only reason why asexual species can be defined. Mutual recognition, which is involved in sex and in social structure, binds local populations into tighter linguistic and genetic clusters than does common descent alone. The cohesive effect of shared signals is seen in the warning colours of mullerian mimics — in *Heliconius* butterflies, regions of hybridization between areas with contrasting signals are narrow, as hybrids are less fit than those giving an unambiguous warning message[18]. A similar mechanism could explain why the boundaries between dialects are so sharp: for example, the pronunciation of some vowels changes over a few kilometres between the English Midlands and East Anglia[19].

What of the future? Increased mobility and the frequency-dependent disadvantage of rare languages mean that linguistic isolates are now endangered species. The diffusion of genes and languages will tend to smooth out barriers. For example, Low and High German are distinct dialects separated by a sharp linguistic boundary across northeastern Germany. In the Rhine delta, which has been settled for much longer, they have become blurred: separate components change over in different places[19]. It is still an open question whether the newly found concordant genetic boundaries that subdivide Europe are just the neutral relics of ancient movements, or whether they are actively maintained by linguistic barriers. Detailed studies of the genetics of frontier populations are needed before we can understand what shaped the nations of the modern world. ☐

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DAEDALUS

Superficial data

In today's increasingly automated world, one problem looms ever larger. How do you prove to a machine that you have a right to do something? It may be paying for a service, drawing money from a cash dispenser, travelling on a bus or train, entering a workplace, logging on to a computer, making a telephone call or many other things. The current solution lumbers us with masses of cards, tickets and so on, often backed up by memorized codes as well.

Daedalus has a new solution: a machine-readable tattoo. A simple barcode on the wrist, say, might identify the wearer, but could never be updated or expanded, and would be very easy to forge or copy. So Daedalus proposes a magnetic tattoo, an area of iron oxide implanted into the skin. Iron oxide can be made in various shades of yellow and brown, cosmetically quite acceptable to most races (whites will be hardest to colour-match). Once in place, the tattoo could be written to, or read from, by standard magnetic methods.

This elegant scheme could encode all sorts of permanent or temporary authorizations. Name, date of birth, social-security number and blood group would be quite permanent. Credit-card data, driving-licence details, workplace status and magnetic pass keys might change from time to time. In addition, the tattoo could be used for all sorts of temporary or rapidly changing data. Its credit balance would alter every time the wearer bought anything with his tattoo, or paid money into his account with it. He could use it as a bus or train or theatre ticket, on which authorization could be written at the pay desk, checked at any time by officials, and erased at the exit. The masses of clumsy cards and tickets which now encumber our wallets would be completely outmoded.

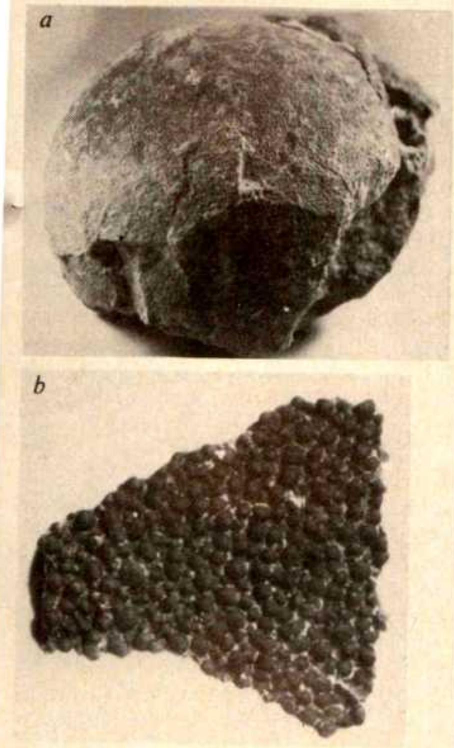
Security would improve, too. You can't lose or steal a tattoo, nor can you easily photograph or copy a magnetic pattern. Even so, crucial financial data might best be protected by an update code. The computer reading or altering this data should always write a new check code on the tattoo and keep a note of it. A forger cannot know the latest code, so his forgery will always be out of date. Accidental erasure will be largely prevented by the intervening barrier of skin; in fact tattoo-coding equipment will need a special high-field writing head.

As the magnetic tattoo gains social and legal status, it may acquire ceremonial value as well. Daedalus has visions of the marriage of the future, in which bride and groom have their marital status ceremonially updated; and the ritual humiliation of bankrupt businessmen or disgraced officials by public erasure of their credit limits or workplace-status numbers.

David Jones

Dinosaur eggs from Romania

SIR—A recent rock fall at an outcrop of Late Cretaceous age (65 million years ago) in the northern part of the Haţeg Basin of Transylvania, Romania (4.5 km east of the town of Vălioara) has revealed the first dinosaur eggs to be found in Romania. Fourteen eggs, distorted to varying degrees, were discovered in a 0.5-m-thick layer of red, silty, clay that has since been found to contain fine volcanic ash and is sandwiched between iron-rich marls, with thin calcrete nodules and tubular burrows beneath. An erosional surface above the clay bed is topped by a thick bed of conglomerate of epi- and volcaniclastic elements in a tufitic matrix.



a, A well-preserved egg attributed to "*Magyarosaurus*" collected from the locality near Vălioara (0.23 × natural size). *b*, External surface of the eggshell at low magnification to show the tuberculated surface (× 1.58).

The eggs are sub-spherical in shape and were found lying clustered in four linear rows, each comprising either two or four eggs. The distance between two clutches containing respectively two and four eggs was 0.5 m. Eight eggs are well preserved, with their lower surfaces almost complete, but the upper surfaces cracked and eroded (see figure). Shell fragments from the upper surface were found in the sediment surrounding the eggs. No skeletal remains were found.

The thickness of the eggshell varies between 2.3 and 2.4 mm. The external surface is irregularly tuberculated (*b* in the figure) and air canals between the tubercles are filled with calcite. The tubercles mark the external ends of densely packed and wedge-shaped, prismatic crystalline units that do not interlock laterally. Under high magnification they can be seen to be made of fine laminae which lie parallel to the external surface of the shell. These structures correspond closely with those described as tubocanaliculate[1,2]. In vertical section the internal surface of the shell is irregular, following the bases of the crystalline units.

The identity of the animals that laid these eggs, as well as eggs of similar age from southern France, is the subject of debate[1,2]: the Haţeg fauna includes ornithopod, ankylosaurian, theropod and sauropod dinosaurs, pterosaurs, crocodiles, turtles, birds and primitive mammals (multituberculates)[3,4]. The large diameter of the eggs (150 mm) excludes as potential layers all except the larger known elements of the fauna: "*Titanosaurus*" (=*Magyarosaurus*) (a sauropod), *Telmatosaurus* (a hadrosaur), and a newly discovered large theropod (D.B.N., D.B.W. and D.G., manuscript in preparation).

It has been proposed[1,2,5] that tubocanaliculate-type dinosaur eggshells were laid by sauropods: on this basis we conclude that these eggs were probably laid by a sauropod dinosaur, and should be referred to provisionally as *Magyarosaurus* eggs.

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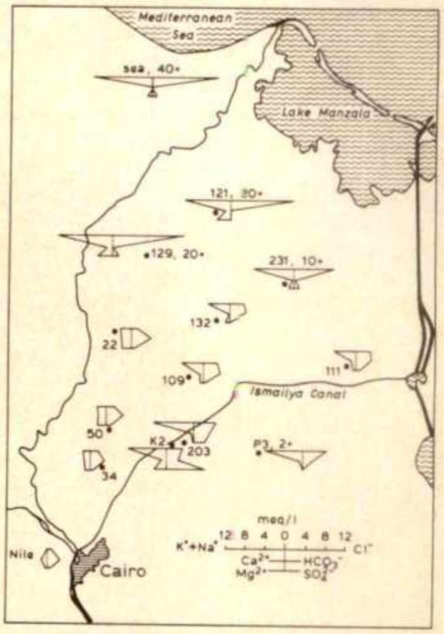
Ground water in the Nile delta

SIR—Burns *et al.*[1] suggest that increases relative to Nile water of the concentrations of sodium and potassium in ground water under Karnak (upper Egypt) may be caused by evaporation, and that the evaporation percentage is 89%, which they consider rather high. The calculation of evapotranspiration from concentration increases in ground water with respect to source water, is an old and simple technique[2,3] that works well when the amounts of the ions are strictly conserved and their sources are known with confidence.

Anions such as chloride are often well conserved because, in many ground waters the only source of chloride is atmospheric deposition; chemical processes do not significantly affect concentration. But cations such as sodium and potassium are typically not conserved, because they are involved in biological cycles, are set free in weathering reactions of feldspars or participate in cation-exchange reactions. The large increase of sodium and potassium reported in ref. 1 could be caused by dissolution of Na-minerals from the soil in combination with cation-exchange.

Fresh ground water in the Nile delta originates almost exclusively from Nile water. Ground water along the Ismailia Canal, which carries water from Cairo to the eastern part of the delta, shows an increase of chloride-ion concentration with respect to Nile water undoubtedly caused by evapotranspiration. At the same time, the ratios of the main cations change, as shown in the Stiff diagrams (see figure). The cations calcium and magnesium show a relative decrease, and sodium and potassium show a relative increase with respect to chloride. The decrease of calcium and concomitant increase of sodium could well be a result of dissolution of sodium minerals such as trona, and



Groundwater compsitions in the Nile delta, Egypt, indicating saltwater upconing in boreholes 121 and 129, and Ca/Na-exchange near the Ismailia canal. Groundwater samples from the archives of the Groundwater Research Institute, Cairo.

cation-exchange in the sodium-containing loess continually blown in from the desert which settles in the wetter delta area. Such effects have been noted in the Negev desert[4], and the coastal plain of Israel[5].

Cation exchange is even more clearly apparent in salt water from boreholes 121 and 129, in which Ca is increased compared with sea water; this is typical of areas where overexploitation of fresh ground water has resulted in the upward movement of salt ground water. The cations from seawater (mainly sodium) then exchange with those from the freshwater sediment (mainly calcium) in a process that is a good indicator of salt water upconing even when detailed hydrological information is lacking[6].

The message is clear: relative increases in groundwater concentrations can be used to calculate evapotranspiration, if the principles outlined by Eriksson are valid. This is difficult to demonstrate for cations such as sodium and potassium.

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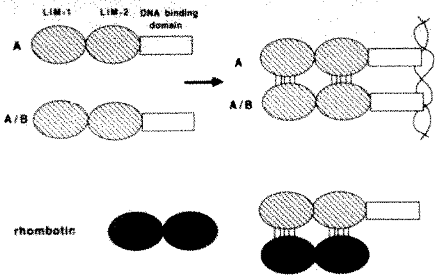
LIM domains

SIR—In the previously reported protein sequence of the presumptive T-cell oncogene rhombotin[1,2], the presence of two tandemly repeated cysteine-rich regions (CRR) was noted, suggesting that these might correspond to metal-binding domains. We have now found that these CRR domains are equivalent to the recently proposed LIM domains in the nematode cell-lineage proteins lin-11 and mec-5 (ref. 3) and the vertebrate transcription factor Isl-1 (ref. 4). This similarity places the rhombotin protein in the family of proteins involved in transcription control. But although lin-11, mec-5 and Isl-1 share a putative DNA binding homeodomain as well as the LIM domains, there is no such DNA-binding domain in the rhombotin protein.

This difference in the putative protein structures, therefore, suggests that LIM domains facilitate, in this case via metal binding, protein–protein interactions in a manner analogous to the leucine zipper[5] or the helix-loop-helix (HLH) motif[6]. Proteins carrying only protein oligomerization motifs could thus compete with transcription factors carrying compatible protein-interaction domains by docking with these proteins and preventing dimerization (see figure). The presence of proteins carrying only protein dimerization motifs may not be without precedent: myoD carries an HLH domain and a basic region thought to be involved in DNA binding[7], and Davis *et al.*[7] cite unpublished evidence for the presence of a protein carrying only an HLH domain in the same cell lineage. Generalizing on earlier proposals[8], interactions of this type could be one way to explain functional inactivation of genes by dominant negative mutations.



Docking model for LIM domain interactions. The hypothetical protein A is capable of homodimer or heterodimer (or oligomer) formation with protein B, via metal linkage at the LIM domains, to form a complex specifically binding to DNA and involved in transcription. The formation of this transcription complex can be competed for by interactions at compatible LIM domains with LIM-only proteins. Activity of such complexes will depend on the balance of concentrations of the various involved proteins. Thus the combinatorial interaction of LIM-only and DNA-binding proteins represents a potential strategy to regulate gene expression.

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Increased bloom

SIR—Algal blooms have recently received considerable attention, particularly when they cause mass mortality of marine organisms. A worldwide increase of algal blooms in coastal waters has been documented and is usually related to man-induced increase in nutrients. It is still open to question whether algal blooms in the North Sea have increased due to such a eutrophication. This debate has considerable political interest: are measures to decrease nutrient inputs to the North Sea necessary?

In the Southern Bight of the North Sea, the colonial flagellate *Phaeocystis* sp. is the most important bloom-forming alga. Fortunately, it is not toxic, but its intensive foam production during the wane of these blooms makes North Sea beaches unattractive during the tourist season[1].

Continuous plankton recorder (CPR) data[2] indicate that *Phaeocystis* around the British Isles decreased from 1948 to about 1970, since when it fluctuated at low levels to increase again slightly from 1980 onwards at the British side of the Southern Bight. For the Dutch side of the Bight the trend is similar, but since 1980 not enough CPR data have been available[2].

This general decline and low levels of *Phaeocystis* after 1970 apparently contradict the almost continuous increase in duration of *Phaeocystis* blooms observed since 1974 at the Marsdiep station[3] for northern Dutch coastal waters. Historical data on *Phaeocystis* in the Marsdiep[4] indicate that *Phaeocystis* blooming periods lasted about 50 days in 1897 and 1899. The duration of these periods has increased two to threefold since then. These historical data and data for 1987–89 confirm our earlier conclusion of an increasing trend in duration of *Phaeocystis* blooms[3].

These two conclusions do not contradict each other: different water masses were sampled in both studies. The towing depth of the CPR (10 metres) prevents any sampling in coastal waters, and the number of sampling routes in the North Sea has decreased. Waters near the Dutch coast have hardly been sampled by CPR, certainly not adequately in recent years.

Dutch coastal waters are eutrophicated by continental river water, particularly from the Rhine[5]. This nutrient-rich water remains near the coast. The change observed during the past few decades in nutrient composition of the coastal waters (increase in nitrogen and phosphorus, no such increase in silica) particularly favours the flagellate *Phaeocystis*[3,5]. Whereas changes in plankton in the open North Sea largely reflect climatic changes[2], in Dutch coastal waters these climatic influences on phytoplankton seem to be overruled by eutrophication effects. Thus, *Phaeocystis* apparently behaves differently in Dutch coastal waters and in open North Sea water, in accordance with the respective elevated and virtually constant nutrient levels.

A decrease in nutrient loadings of the coastal waters is necessary to curb the ever-increasing algal blooms. Apart from nuisance due to foam accumulation on the beach, it is predictable that decomposition of the increased amounts of algal organic matter will lead to oxygen deficiencies, even in normally well-mixed Dutch coastal waters like the Wadden Sea.

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Water balance in the Black Sea

SIR—Murray *et al.*[1] suggest that the combined pycnocline/chemocline in the Black Sea has risen unexpectedly and at an alarming rate. Their argument rests on the comparison of the RV *Atlantis* II hydrographic station 1445 of March 1969, to the *RV Knorr* June 1988 station BS3-2, about 30 nm away in the western Black Sea basin. The two records showed a 0.1‰ increase in salinity between a depth of 50 and 100 m and a rise of the chemocline by 30 m. Murray *et al.* suggest that the water balance of the Black Sea has been upset by a decrease in discharge of Soviet rivers. To compensate, the input of Mediterranean water through the Bosphorus should have been higher. Indeed, a rough calculation of the water and salt balance based on this comparison does not exclude such an interpretation.

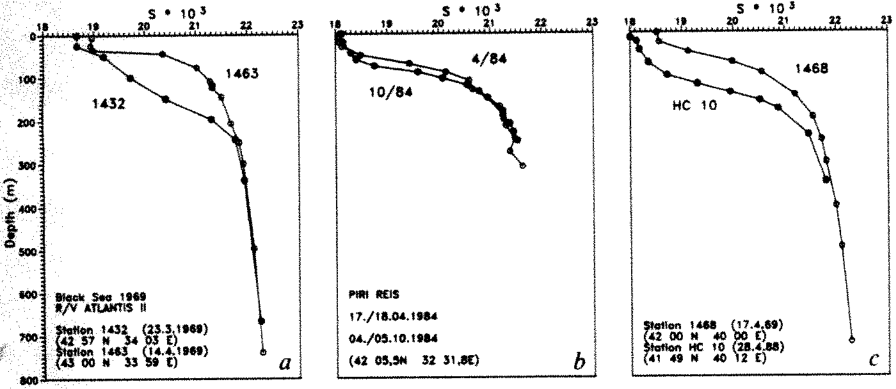
This interpretation, however, may be countered by field observations. For examples, stations Atlantis 1432 (23 March 1969) and 1463 (14 April 1969) were just a few miles apart but showed within three weeks a rise in the salinity profile of up to 80 m between 20 and 220 m depth, that is, a much larger rise than the one reported by Murray *et al.* (*a* in the figure). Second, our salinity measurements during the RV Piri Reis Black Sea cruises of 1984 show a rise of the pycnocline of 15 to 20 m between April and October (*b*). Third, during leg 1 of the *Knorr* Black Sea cruise (16 April–9 May 1988)[2], many conductivity–temperature–depth (CTD) stations were occupied (using the same Seabird CTD as Murray *et al.* and an independent instrument) throughout the entire southern Black Sea.

Nine of these stations can be compared with nearby former Atlantis stations. Seven of them show a higher interface in 1988 than in 1969, but two stations from the eastern Black Sea show a much deeper interface (*c*). Thus the interface seems to be intensively warped, possibly more pronounced in April 1988 than in March–April 1969. A net long-term rise cannot, however, be ascertained, at least not without mapping the interface throughout the entire basin simultaneously. Finally, we also occupied stations near the coast twice within a few hours and observed that the pycnocline moved up and down by several tens of metres between casts. These movements warp the chemocline along the coast and are associated with features of the coastal currents or with internal waves travelling along the pycnocline.

Variations in chemo- and pycnocline depths are strongly coupled. Our measurements of dissolved phosphate in May 1988 show upward movements similar to those observed by Murray *et al.* in the western Black Sea. In the eastern basin a depression of about 30 m was found relative to that at *Atlantis* station 1463. The structure of the manganese particle layer, which is found at the +100 mV level in the redoxcline (and associated with a density of 16.1–16.3$\sigma\theta$) may be indicative of the prevalent transport processes. The layer is deeper, more intense and thicker near the coast. It is often split into several sheets, each measuring a few metres in thickness only.

These sheets may represent layers of the water advecting laterally with different velocities. They could transport cold intermediate water towards the centre of the gyres causing a rise of the pycnocline offshore while at the same time warm and low-salinity surface water is displaced from the centre towards the margins of the Black Sea. Such a change could come about by more intensive coastal surface currents triggered by winter storms. This model would also fit the observations, suggesting laternal transport of resuspended shelf sediments within the upper 200 m of the water column and indicating radical changes in the annual vertical particle flux in the upper part of the water column[3,4,5].

We therefore believe that the observed differences between 1969 and 1988 are associated with interannual changes in the intensity of Black Sea surface currents and with internal waves rather than with anthropogenic changes of the Black Sea water balance.



a, Comparison of salinity between two RV *Atlantis II* stations showing the rise of the pycnocline by up to 80 m within about 3 weeks. *b*, Comparison of salinity profiles of RV *Piri Reis* cruises in April 1984 and October 1984. *c*, Comparison of salinity profiles (Atlantis 1458 with Knorr BS1 HC 10).

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Random thoughts

SIR—A minor slip in Hubert P. Yockey's most informative letter "When is random random?" (*Nature* **344**, 823; 1990) requires clarification. He writes: "Thus it is clear that pi and all other transcendental numbers have a finite amount of information, namely, that in the algorithm by which they are calculated." From this statement one would infer that every transcendental number may be calculated by some algorithm. But there are only countably many algorithms and uncountably many transcendental numbers. Hence one cannot conclude from Yockey's argument that each transcendental number contains only a finite amount of information. This issue is, of course, only incidental to the point of his letter.

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SIR—The discussion about the meaning of 'random' needs to take into account two separate uses of the word. Information-theoretic or algorithmic approaches to measuring the randomness in a sequence of digits may be appropriate after the sequence has been generated or defined, but there is also a sense in which a process may be agreed to be random before it has been used to generate any sequences whatever. A sequence produced by a random process is not necessarily random.

Yockey (*Nature* **344**, 823; 1990) blurs the distinction by using the name 'Bernoulli chains or strings' for the actual sequences of heads and tails (say) generated by what probabilists have long called 'Bernoulli trials'. It is not necessarily true that '[i]n the case of a sequence of heads and tails generated by the tossing of a fair coin, there is no algorithm which can shorten the sequence' because the sequence might be all heads, for example. Bernoulli's *Ars conjectandi* contained no discussion of actual sequences, and his name should surely be reserved for the generating process.

But, as Yockey goes on to say, "It is impossible to prove that any given sequence was generated by a random process"; in other words, it is impossible to prove that a process is random by examining its output sequence. Rather, a process is called random if its structure ensures that its output is unpredictable.

Statisticians are familiar with the separate uses. A good experimental design will need to be based on the first kind of randomness lest it display systematic bias, but a randomized trial will need the second kind so that the participants cannot guess what is coming next.

Whether randomness can be measured is a difficult problem (*Nature* **344**, 705; 1990). One cannot judge the absence of pattern without specifying which pattern, and what is a pattern to you may not be a pattern to me.

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Superconductor defect structure

SIR—One of the main obstacles to the practical use of high-temperature superconductors is that the amounts of current that they can carry and still remain superconducting (that is, their critical current densities) are relatively low. A processing method that has significantly increased the intragranular critical current density involves the solid-state phase transformation of single-phase $Y_2Ba_4Cu_8O_{16}$ (commonly referred to as '124' or '248') into $YBa_2Cu_3O_7$ ('123') with the concomitant formation of a heavily defective microstructure. Here we report a defect structure of apparent composition $YBa_2Cu_5O_9$ ('125') in solid-state-transformed sintered bulk samples of 124.

The decomposition of 124 precursor was carried out by heat treating it at 930 °C for 1.5 min followed by a low temperature oxygenation process of rapidly cooling (10 s) to about 750 °C with further cooling to 380 °C at 20 °C h^{-1} in flowing oxygen. Further details of sample preparation are given elsewhere[1]. The defect structure was examined by high-resolution transmission electron microscopy (HREM) using the Berkeley atomic-resolution microscope. Thin foils were prepared by mechanically grinding a slice of the superconductor to about 50–80 μm, followed by argon-ion milling to electron transparency at 6 kV and 77 K. Images were obtained under conditions close to Scherzer defocus and the projections of atomic columns therefore appear as dark dots on a white background. To avoid electron-beam-induced damage, images were typically recorded within 2 min of exposure to the beam.

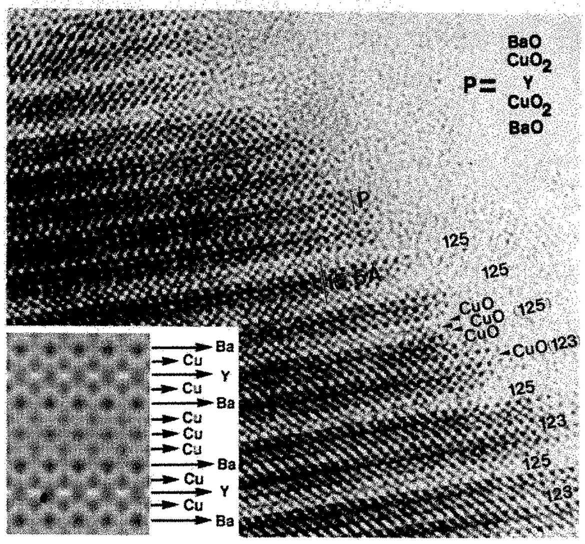

FIG. 1 Atomic-resolution image of the phase-decomposed 124 sample illustrating the 123 structure alternating with the new 125 defect structure. Layers of 125 two or three unit cells thick are seen in the upper and middle part of the micrograph. The simulated intensity distribution for the cation positions (inset) of 125 for a foil thickness of 12 Å and an objective-lens defocus of −500 Å, confirms the interpretation of the contrast in the micrograph.

A micrograph of the defect structure (Fig. 1) shows the decomposition of 124 into 123 and 125. The structures of the three related phases are illustrated in Fig. 2 (*a*–*c*). Analysis of the image contrast using detailed image simulations indicates that the basic perovskite building blocks (marked P in Fig. 1) in the 125 structure are similar to those in the 123 structure, except that the 125 structure has three CuO chain layers whereas 123 has one. The 124 structure has two CuO chain layers with an *a*/2[100] glide plane between them[2,3]. In the proposed 125 structure (most likely $YBa_2Cu_5O_9$) the three CuO chains are mutually related by a 1/2[100] glide operation and hence, unlike 124, there is no doubling of the unit-cell dimension along the *c* direction. The *c*-axis lattice parameter is expected to be 15.6 Å, based on the addition of one more CuO chain layer to the 124 structure (the Cu–O distance along the *c* axis is about 1.9 Å, as established by neutron diffraction and HREM analysis[2]). In general, single layers of 125 alternate with single layers of 123 but occasionally layers of 125 two to three units cells thick occur.

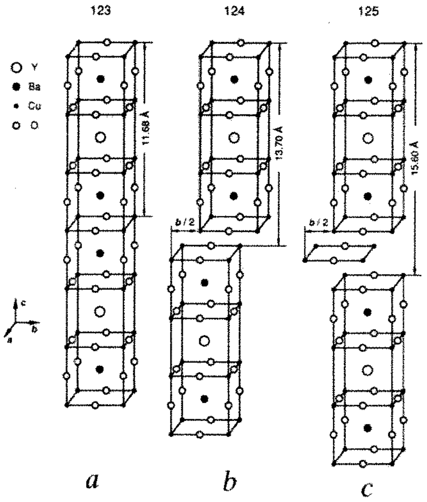

FIG. 2 Schematic illustrations of the structure 123, 124 and 125.

The fact that the 123 and 125 layers are formed as alternating layers with very rapid kinetics suggests that the early stage of the decomposition of 124 may occur by a spinodal (spontaneous decomposition without a nucleation barrier) process. Such a decomposition to 123 requires only short-range diffusion along the *c* direction (jumps over atomic-layer distances), as can be seen from Figs 1 and 2. During the later stage of decomposition, however, nucleation and growth reactions seem to take over, involving the removal of copper and oxygen by long-range planar diffusion to form equilibrium, twinned 123 and spherical CuO inclusions.

So far, our attempts to synthesize the 125 phase as a single phase in bulk form by conventional ceramic processing have failed. Perhaps the formation of the 125 phase is easier when the 124 phase spontaneously decomposes into the 123 structure, forcing the formation of the 125 structure. We note that recent three-dimensional Monte Carlo simulations using experimental thermodynamic data predict that a bulk 125 containing material can be synthesized at a very high oxygen pressure in a copper-rich environment (M. Fendorf, personal communication).

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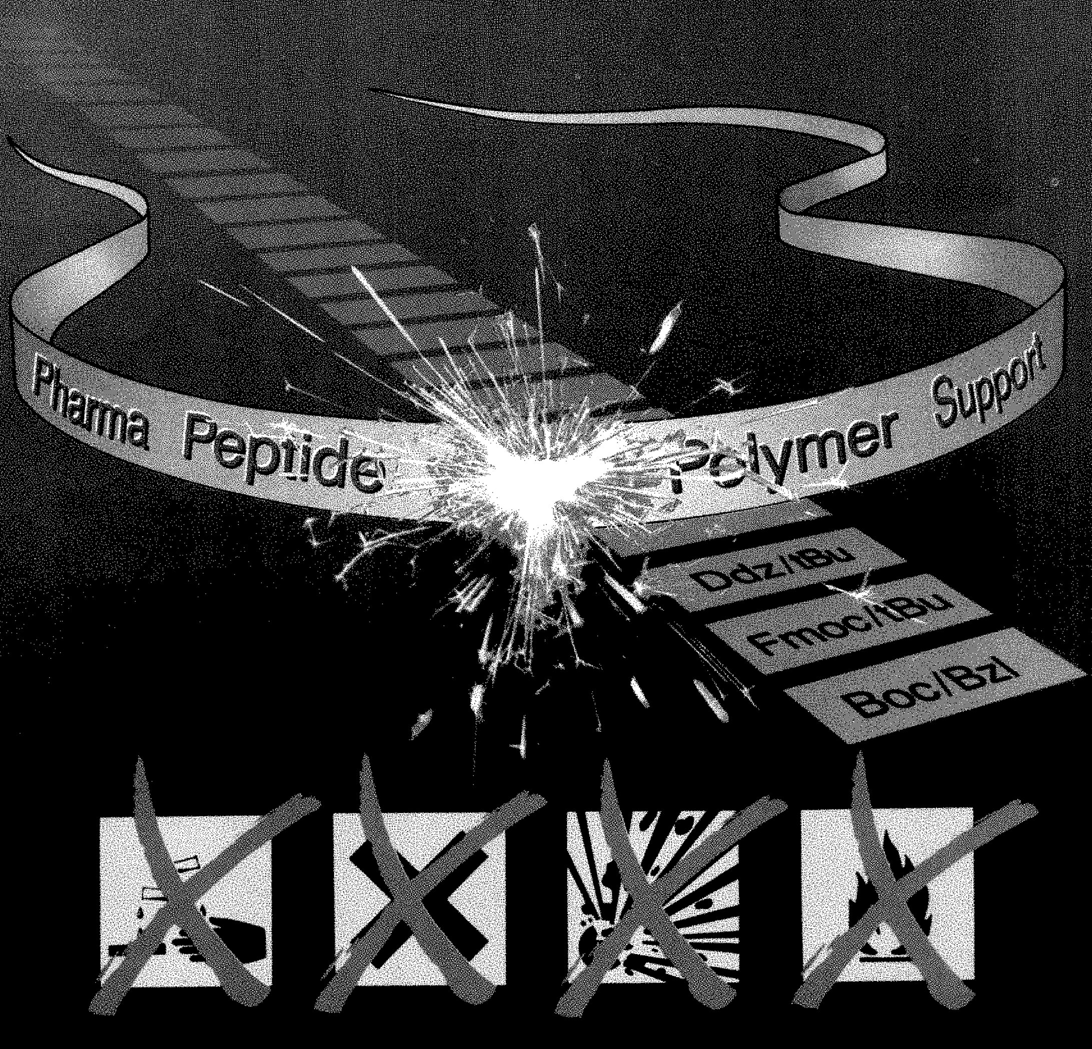

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Diversity and differences

Henry R. Bourne

Transmembrane Signalling. Intracellular Messengers and Implications for Drug Development. Edited by Stefan R. Nahorski. *Wiley: 1990. Pp. 248. £39, $76.*
G-Proteins as Mediators of Cellular Signalling Processes. Edited by M. D. Houslay and G. Milligan. *Wiley: 1990. Pp. 232. £35, $72.45.*

TRANSMEMBRANE *Signalling* presents papers from a meeting held in the spring of 1989. As a symposium volume, it displays all the distinguishing marks of the genre. It is out of date. It promises more than it delivers. It neglects fundamental questions in favour of reciting facts collected in individual laboratories. More specifically, of fourteen chapters, nine are devoted to a single mode of transmembrane signalling, via G proteins and their confederate receptors and effectors; the remaining five focus on a single network of intracellular messengers, the Ca^{2+}/phosphoinositide cascade. The book's title suggests that it might have accorded to receptor tyrosine kinases more than the offhand comment in chapter 8, and to cyclic nucleotides more than the four pages on synthetic cyclic AMP analogues in chapter 14. Moreover, its pages provide not a single example of the "implications for drug development" advertised in the subtitle.

False advertising might be forgiven if the product were more timely or more interesting. Most chapters, however, contain little more than accounts of the research of one laboratory, about two years ago. Of the book's first 234 references (after which I stopped counting), only five cite papers published later than 1988. Sadly, detailed findings of a single laboratory — even a good one — do not become more fascinating as the years pass. In mid-1990, only a few chapters — including those by Birnbaumer *et al.* (on G proteins and ion channels), Irvine (inositol polyphosphates) and Pouyssegur *et al.* (G proteins and cell proliferation) — are sufficiently thoughtful, provocative or comprehensive to merit attention. Note, however, that more recent reviews of the same subjects, in some cases by the same authors, are available at much lower cost, by taking the time to peruse a few current journals on library shelves.

Rather than embalming a venerable symposium, the editors of *G-Proteins as Mediators of Cellular Signalling Processes* have put together the first in a projected series of scholarly multi-author books on the "molecular pharmacology of cell regulation". In this case the book's title correctly describes its contents, which ought to be relevant to many biologists in an era when one can hardly open a journal without meeting GTP. Several chapters provide exactly what one seeks in a short monograph. Hall comprehensively surveys recent literature on Ras proteins and their relatives, making more than the usual amount of sense out of a plethora of diverse and sometimes contradictory experimental observations in many laboratories. Hingorani and Ho do the same for the well-studied G protein of retinal photoreceptors, transducin; their bold representations of relations between transducin structure and function will provoke thought and experiment, even if some predictions turn out to be wrong.

In general, however, the book is not appreciably more up to date or comprehensive than the symposium volume. Again, references after 1988 are rare, and too many chapters concentrate on recounting experimental findings in their authors' own laboratories. Self-citations account for more than a quarter of the references in both books. The narrow focus characteristic of symposium contributions is especially inappropriate in scholarly summaries of a complex field.

G-Proteins would have profited from a more assertive editorial hand. The editors should have corrected obviously erroneous statements (for example, the attribution, on page 1, of a single transmembrane region to catalytic adenylyl cyclase), as well as the typographical errors found on almost every page (such as the "autonomic" functions of transducin, on page 101). They should have deconvoluted pompous, obscure prose in several chapters (see page 86 for a rich assortment of abominable sentences). They should have blue-pencilled the boring, repetitious accounts of G proteins that introduce every chapter. Finally, far too many chapters portray a motley army of lonely facts — gel filtration profiles, immunoblots, amino-acid sequence motifs and graphs of something versus something else — wandering mindlessly across the page in search of an idea. The editors could have produced a much more interesting and readable book.

Every scientist can name at least one collection of scholarly reviews (even, perhaps, a symposium volume) that challenged active investigators, recruited new ones and enriched a field. But in many fast-moving research areas, the quality of most such tomes is negligible and likely to remain so. The root of the problem is not greedy publishers but scientists themselves. Some of us covet cheap air travel, paid for with a manuscript, while others just yearn to see our names in print. Who among us has not scribbled a rehash of past exploits without bothering about whether anyone will (or should) read it? We have met the enemy, and he is us. □

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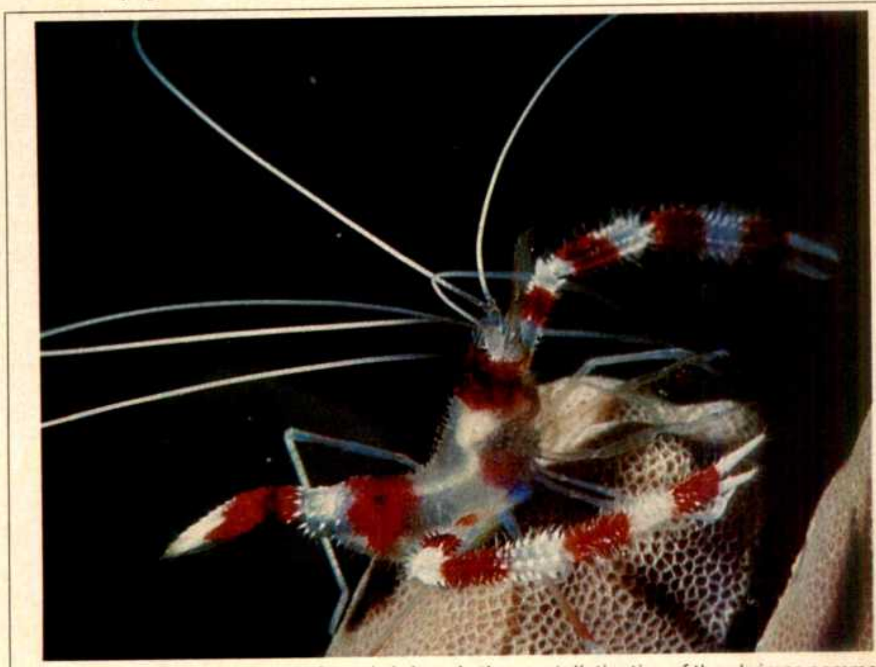

Stenopus hispidus, the banded coral shrimp, is the most distinctive of the shrimps common to the reefs of Hawaii. Taken from *Hawaiian Reef Animals* by E. Hobson and E.H. Clave, first published in 1972 and now reissued in a revised edition. The volume describes the vertebrate and invertebrate communities of the Hawaiian reef, and contains 85 colour plates of the variously decorated animals of the reef. Published by University of Hawaii Press, pbk $19.95. □

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- *Stars and Galaxies. Citizens of the Universe* edited by Donald E. Osterbrock is a recent addition to the series "Readings from Scientific American". The collection presents research on galaxies, luminous stars and supernovae. Publisher is Freeman, price is £10.95.
- *The Path of No Resistance* is subtitled *The Story of the Revolution in Superconductivity*. Published by Simon and Schuster as a Touchstone paperback, the author is Bruce Schechter, price is $8.95. ☐

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Seeds of the past

Benno Müller-Hill

The Wellborn Science. Eugenics in Germany, France, Brazil and Russia. Edited by Mark B. Adams. *Oxford University Press: 1990. Pp.242. £35, $45.*

For a long time, eugenics has been forgotten by historians of science. Now, when almost all the participants are dead — and just before the Human Genome Project becomes a reality — their interest in the subject is re-emerging. So far, all the books on the subject have concentrated on eugenics in just one country. This one, edited by Mark Adams, differs in that it is the first which attempts an international approach. Four authors deal with four countries: Germany, France, Brazil and the Soviet Union. Four authors are necessary, according to Adams, because scholars in the United States know at best one language in addition to their own. So the approach is necessarily fragmented. Nevertheless, only one-tenth of the countries in which eugenicists were active are dealt with here.

The difference between what has been called eugenics in each of these four countries is tremendous. On one end of the scale is Brazil, where not a single researcher ever actually worked on genetics until 1940, the year the study ends. Eugenics in Brazil was a mixture of sport and hygiene. Nobody there ever thought seriously about sterilization, and nobody was upset by race mixing — everybody optimistically expected the "whitening" of the inhabitants. The article by Nancy Leys Stepan confirms what one may have guessed: eugenics in the absence of geneticists cannot have a strong impact on society.

Strangely enough, according to William Schneider, France was not so very different from Brazil. Before World War I eugenicists in France were inspired more by Lamarck and by Zola's novels than by Mendel. According to Schneider only one geneticist, Cuenot, was active in France before 1930. But the ultimately hopeful world of Zola collapsed with World War I, and a new generation of eugenicists appeared in the 1930s. Curiously, Schneider does not mention the French Nobel prizewinner Alexis Carrel, who proposed the "ideal solution" of "little economic gas chambers" for all those with bad genes in his book *L'homme cet Inconnu* (Plon, 1935). Schneider's account stops at the moment where it would have become the most interesting with the collaboration between the French and the Nazi authorities in 1940. So the Swiss anthropologist Montadon is mentioned as "never subscribing to the possibility, let alone the advantage of achieving racial purity". Montadon certainly changed his opinion in his tract "What is a Jew?", which was published during the German occupation and has just been reprinted in France.

Adams paints a fascinating picture of the development of eugenics in the Soviet Union, where the subject was strongly associated with some outstanding mendelian geneticists. Kol'tsov, Filipchenko and Serebrowsky understood and practised genetics. Kol'tsov, who wrote in 1922 "Eugenics is the religion of the future and it awaits its prophets", was an optimist who could not have imagined somebody like Hitler. To propose mass sterilization was out of the question in this devastated country, so Serebrovsky, and later H. J. Muller, proposed sperm donation. But with the rise of Stalin, genetics and eugenics disappeared with the geneticists.

The other country considered here in which geneticists were strong and eugenics was flourishing, Germany, is dealt with by Sheila Weiss. Her article has to compete with Weindling's voluminous book *Health, Race and German Politics 1870–1945* (Cambridge University Press; for review see *Nature* **344**, 502; 1990) and does so very well. Germany was the only country where eugenical measures were applied on a vast scale by the government. Weiss concentrates on the different aspects of the various breeds of eugenics. The details of the Nazi variety as propagated by various state agencies are somewhat neglected. But she shows convincingly how just one brand of eugenics was selected by the Nazis and brought into action.

At the end of the book the reader is left with some nagging questions: why was eugenics so different in different countries? Why were eugenicists successful only in Germany? Could some other form of the practice become successful in the future? The reader is reminded by Adams in the concluding chapter that the book deals after all with only four of about forty countries in which eugenicists have flourished. And if the past is so complex, what about the future? I think this volume should be bought and read not only by historians of science but by all those interested in and involved in the Human Genome Project. To know what was thought and what happened in the twentieth century may be helpful for those who will be active in the twenty-first. ☐

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- In his last book review for *Nature* (**336**, 721; 1988), Professor Müller-Hill reviewed *The Bison* by Danil Granin, a novel about Nikolai Timofeyev-Resovsky, the scientist who defied Stalin. Just translated into English by A. W. Bouis, the book is now published in the United States by Doubleday, price $24.95. ☐

Business plan

Bob Johnstone

Computers Inc. Japan's Challenge to IBM. By Marie Anchordoguy. *Harvard University Press: 1990. Pp. 273. £19.95, $25.*

INDUSTRIAL policy can work. That is the inescapable conclusion of this important new study on the rise of the Japanese computer industry. Classical economic theory — as preached by zealots of the Bush administration — states that governmental attempts to 'pick the winners' inevitably distort the market, ending up by doing more harm than good. Yet, as the Japanese have demonstrated, and as this book documents, an industry can indeed be nurtured and brought to international competitiveness. What it takes is a consistent, long-term strategy and close cooperation between public and private sectors. Proof, if proof is required, of Japan's success in the computer industry is evident in the admission by Ed Lucente, president of IBM's Asia–Pacific group, that IBM views Japanese computer companies as "our most serious competitors".

In 1960, IBM obtained permission from Japan's Ministry of International Trade and Industry (MITI) for permission to manufacture in that country. Back then, only brave souls would have predicted that, within two decades, Japanese domestic manufacturers would catch up with and — at least in some respects — overtake the giant US firm.

But that is exactly what happened. In 1980, Fujitsu — Japan's leading computer manufacturer — became the first company in the world to top IBM in computer sales. Although IBM remains the dominant force in the worldwide computer industry, with an estimated 70 to 80 per cent share of the $40,000 million mainframe market, the Japanese are eroding the US company's market and technological leadership (see last week's News section, page 307). In June this year, for example, Hitachi, Japan's other maker of IBM-type mainframe computers, announced that it would shortly begin sales of machines considerably faster than IBM's current top of the range models. Less than a month later, Hitachi's rival NEC produced a mainframe that it claims is even faster than Hitachi's.

The Japanese are adept at using the threat of foreign dominance — real or imagined — to motivate themselves to redouble their efforts in any given endeavour. Of all the bogeymen they use, IBM has traditionally been the most frightening.

IBM's request for permission to produce locally seems to have reminded MITI bureaucrats of the arrival in Tokyo Bay, about 100 years earlier, of Commodore Perry with his fleet of black ships, demanding that Japan open up to foreign commerce. MITI officials determined that the giant US firm would not be allowed to dominate the Japanese market as it had — in their estimation — in Europe. To counter IBM, says Anchordoguy, "Japan would turn to its full repertoire of industrial policy tools". These included protectionism, appeals to patriotism and outright coercion. Fiscal tools included subsidies, tax-benefits, low-interest loans and loan guarantees.

It is often thought that the Japanese government merely sprinkles financial aid on infant industries, leaving companies to foot most of the bill. In the case of the computer industry, however, as Anchordoguy is at pains to point out, this was certainly not true. "A conservative estimate shows", she writes, "that some US $542 million in subsidies, tax benefits, and loans were granted to the industry from 1961 to 1969." This figure does not include "the huge benefits of procurement from NTT" — Japan's public telecommunications monopoly, which played a very important complementary role in the development of the Japanese computer industry — "and other government institutions."

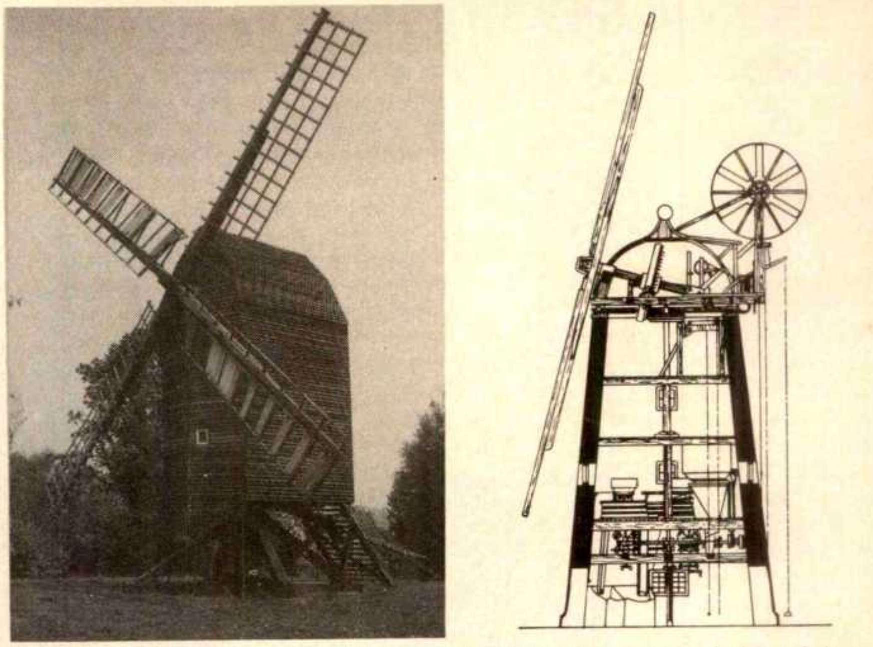
Perhaps the most significant, and certainly the most innovate, support mechanism that MITI came up with to boost sales of computers was the Japan Electronic Computer Company. This was a government-funded company which between 1961 and 1981 purchased computers worth $2,000 million from the makers, then rented them to users. The company had the dual purpose of providing companies with cash that they could quickly plough back into development, while at the same time stimulating demand by making computers relatively easy for users to afford.

But MITI also made some false moves. Most notable among them was the Japan Software Company, a joint venture it created to provide programmes for computers developed under a ministry project. Isolated from the market, the firm failed to produce software that anybody wanted, and was dissolved in 1972.

For the most part, though, things went according to the ministry's master plan. One important factor that helps to account for the success of MITI's strategy was that it managed to achieve a workable blend of cooperation and competition between firms. Japan was thus able to avoid the pitfalls of the single-company 'national champion' approach of Britain and France.

Companies were happy to make the best use of scarce resources by collaborating on upstream development efforts. Nor did they object too strenuously to being paired off (Fujitsu with Hitachi, NEC with Toshiba and Mitsubishi with Oki) to attack different market segments, thus avoiding excessive competition. In their individual segments, however, companies competed fiercely against one another. And, despite generous government subsidies, all were prepared to commit significant investments of their own.

Others have told the story of the development of the Japanese computer industry before. But no-one has told it so well, or in so much detail, as Anchordoguy in this excellent book. □



Early windmill design, from *The Traditional Buildings of England* by Anthony Quiney. Published by Thames and Hudson, price £14.95. □

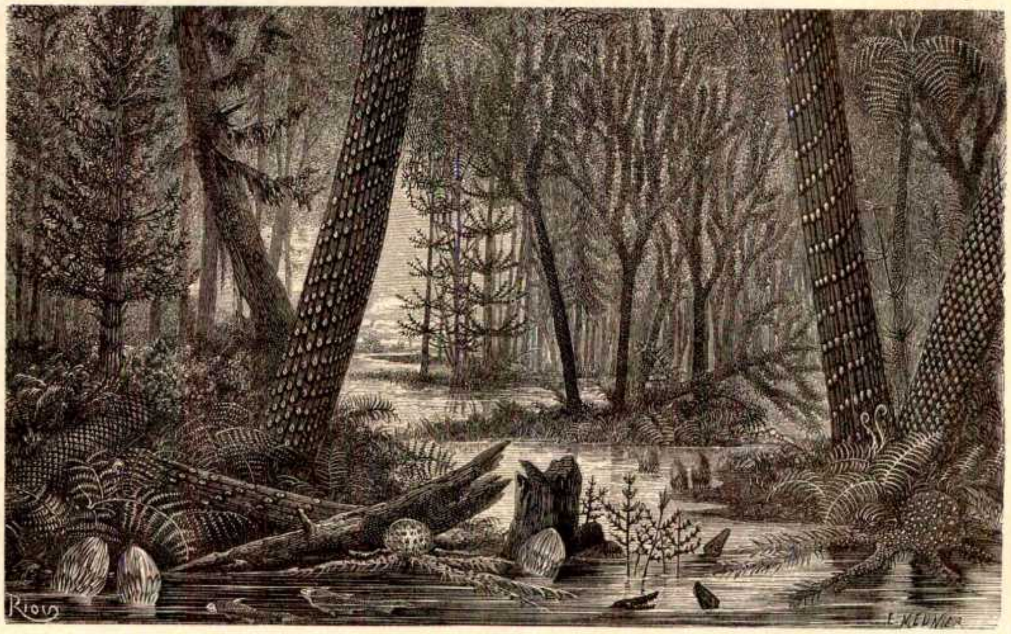
Bob Johnstone is Technology Correspondent of Far Eastern Economic Review, *Centre Point, 181 Gloucester Road, Hong Kong.*

View from the shore

Simon Conway Morris

Terrestrial Invasion. An Ecophysiological Approach to the Origins of Land Animals. By Colin Little. *Cambridge University Press: 1990. Pp. 304. Hbk £40, $69.50; pbk £13.95, $29.95.*

ONE of James Lovelock's most compelling images is that of the early Earth, not as an azure jewel hanging in the blackness of space, but a dirty red smog-bound ball. Such invocations haunt us because they provide the mainspring to catapult our imaginations into the distant geological past. How, then, would a time-traveller see our planet a mere 450 million years ago, towards the end of the Ordovician? An atmosphere now cleansed by the purifying action of oxygen, deep blue oceans and polar ice-caps, but the continents themselves dun and barren, devoid of plants — except, perhaps, along the fringes of the sea, where a green tideline heralded a biological revolution. The invasion of land was on, and here is a scholarly book to guide us as to how this might have been achieved.



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Historical view of animals' attempts on the land.

It is a story that demands grand themes, but the reader will have to work pretty hard to find them among all the minutiae. Most striking, yet most neglected, is just how dynamic terrestrial invasion has been. For too long it has been construed as a largely Palaeozoic affair. The evidence is, however, that some animals were creeping onto land much later, in the Cretaceous or even in the Tertiary. These animals might have included the decapods and littorinacean snails. Indeed, the evolution of entire shoreline communities, especially the mangrove swamps and saltmarshes in the Cretaceous, may have done much to facilitate successful terrestrial transfer as well as those making the return journey to the sea. Ecological analogues to the mangrove swamps earlier in geological history are decidedly tenuous, so here is a timely warning about the inapplicability of uniformitarian ecology.

Meanwhile, the invasion of land probably continues. Little's suggestion that diversity of a group could be linked to time of invasion might mean that the taxonomically depauperate terrestrial amphipods may have come ashore only in the past few million years. Moreover, many of the stages of terrestrial invasion are still evident from the varying abilities of shore-dwellers to cope with the rigours of the subaerial habitat. In some, relative success is marked by physiology, but simple rules of thumb are elusive and factors such as behaviour are often significant.

Another theme is to identify the main routes of invasion. Great stress is laid on physiological evidence. For example, terrestrial groups with either osmotic tolerance or high osmotic pressure are taken to indicate a direct marine origin. There are some alarming exceptions. In teleost fish, blood composition indicates a freshwater phase, although why marine representatives still carry this apparent signature is far from clear. The book is peppered with such examples of wayward ecology and physiology where no satisfactory explanation exists, and although Little is properly cautious there is a sense of the *ad hoc* about some proposals. Take also the potentially fascinating cases of failures in the invasion of land. For no obvious reason the gecarcinid crabs failed to penetrate inland, and despite their adroit subaerial excursions, the mudskippers apparently have not given rise to terrestrial lineages.

Little has much of value to say on the environments of invasion. He argues that beaches were a plausible route, with the severity of the transitional zone acting as a testing ground for would-be terrestrial aspirants. In the case of the vertebrates he casts further doubt on the time-honoured notion of proto-amphibians lurching from one drying pool to another, and suggests our terrestrial origins lay in stagnant freshwater swamps where oxygen crises imposed the need to switch to air-breathing.

This book is a rich lode of information, and it will be a shame if evolutionary biologists overlook some of its treasures. In particular, it represents a marvellous quarry for students of convergence as group after group grappled with the tremendous changes that assault the terrestrial invader. These are most striking in terms of respiration, take for example the remarkable 'lung' of the crab *Ocypode*, and sensory perception as emphasis shifted from chemical to visual cues. But what is urgently needed is a phylogenetic perspective to this welter of ecological and physiological data. The fossil record may be appalling (although, curiously, Little does not mention the amazing finds such as those from the Devonian deposits at Gilboa), but detailed phylogenetic testing is still feasible.

So here is an excellent compilation which, if somewhat naive in its geological sections, is unrivalled in its breadth and gathering of examples. It represents serious scholarship, and will be a source to all who seek to understand how it is that the land teems with life. But the main themes in terrestrial invasion, those that must inspire the next generation, still await their biological Boswell. ☐

Simon Conway Morris is in the Department of Earth Sciences, University of Cambridge CB2 3EQ, UK.

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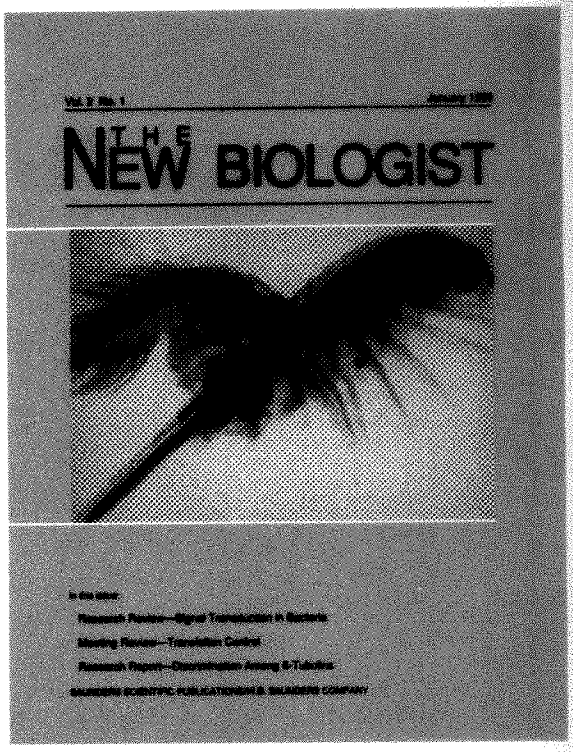

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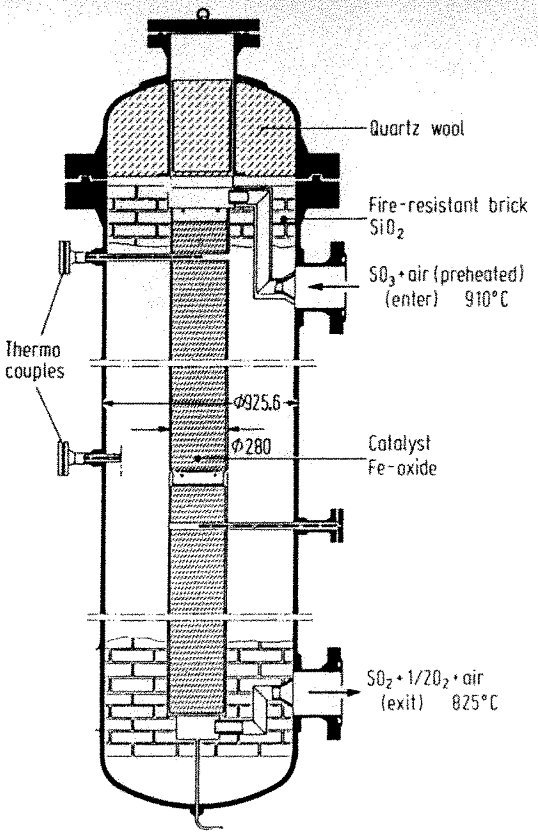

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Adhesion receptors of the immune system

Timothy A. Springer

The adhesive interactions of cells with other cells and with the extracellular matrix are crucial to all developmental processes, but have a central role in the functions of the immune system throughout life. Three families of cell-surface molecules regulate the migration of lymphocytes and the interactions of activated cells during immune responses.

THE organization of animal cells in differentiated organs and tissues has long been postulated to depend on cell-surface interactions both with molecules on the surface of other cells and with the extracellular matrix[1,2]. Localization of cells can thus in principle be driven by the interplay between interactions with cell-surface and matrix molecules that regulate adherence, and chemoattractant gradients that direct cell migration. These principles operate throughout the biology of multicellular organisms[2,3], and the cells of the immune system in particular depend upon regulated interactions with other cells to activate and direct the response to infection. The distinctive antigen-specific interactions studied by immunologists, and the cell and tissue-selective adhesive interactions studied by cell biologists, have however traditionally found little common ground. This has changed recently as studies of the interactions of T lymphocytes with antigen-bearing cells have yielded not only the antigen-specific receptors, but also a harvest of cell adhesion molecules. Homologous families of adhesion receptors reveal relationships between molecules in the immune system and many other tissues, and participate in the control not only of cell–cell interactions but also in the regulation of cell migration.

The mechanisms for regulating adhesion, and their functional importance, are particularly richly illustrated by the adhesion receptors of the immune system. To patrol the body effectively for infectious organisms, the cells of the immune system must both circulate as nonadherent cells in the blood and lymph and migrate as adherent cells through tissues; in the presence of a foreign antigen, they must be able to congregate in lymphoid organs, cross endothelial and basement membrane barriers to aggregate at sites of infection, and adhere to cells bearing foreign antigen[4]. Rapid transition between adherent and nonadherent states is of key importance to the dual functions of immune surveillance and responsiveness. In this review, I shall explain what is known of the adhesive interactions that take place when lymphocytes have been activated by foreign antigen and that direct their localization and migration, before describing receptors that determine lymphocyte homing to different lymphoid organs and neutrophil localization in inflammation. Three families of adhesion receptors mediate these interactions (Figs 1–3): the immunoglobulin superfamily, which includes the antigen-specific receptors of T and B lymphocytes; the integrin family, which is important in dynamic regulation of adhesion and migration; and the selectins, which are prominent in lymphocyte and neutrophil interaction with vascular endothelium. Many adhesive molecules play a part in more than one of these functions, and some of them are exploited as receptors by viruses.

Antigen-specific recognition by T lymphocytes

Most of our knowledge about the molecules regulating lymphocyte adhesion has come from the study of T lymphocytes, whose functions in immunity depend on close contact with other cells. The T-cell receptor (TCR; Fig. 1) recognizes antigen as a peptide fragment bound to cell-surface molecules encoded by the major histocompatibility complex (MHC; Fig. 1). T lymphocytes are activated by specific binding of their receptors to peptide–MHC complexes on the surface of antigen-presenting cells such as macrophages. This stimulates proliferation and differentiation of two types of functional T cells: helper cells, which promote the proliferation and maturation of antibody-producing B lymphocytes; and killer cells, which lyse cells infected with intracellular parasites such as viruses. Helper T cells recognize MHC–antigen complexes on the surface of the B cells whose proliferation they induce; killer T cells recognize complexes of viral peptides with MHC on the surface of the infected cells they kill.

The MHC molecules that present peptide antigens for recognition by TCRs fall into two structurally distinct classes (Fig. 1). MHC class I molecules bind to peptides derived from endogenously synthesized molecules such as viral proteins in infected cells and are primarily recognized by killer cells (Fig. 4*a*). MHC class II molecules bind to peptides derived from endocytosed antigen, and are recognized primarily by helper T lymphocytes (Fig. 4*b*).

All of the molecules now known to participate in the recognition by T cells of their targets were originally identified by monoclonal antibodies against cell-surface molecules of T lymphocytes. Two of the molecules so identified, CD8 and CD4, have since been shown to act as co-receptors respectively for class I and class II MHC molecules[5,6]. The T-cell receptor and the co-receptors diffuse independently in the plane of the T-cell membrane until they are brought together by co-recognition of the same peptide–MHC molecule complex (Fig. 4*a*, *b*). At physiological densities on T lymphocytes and in the absence of antigen, CD4 and CD8 mediate little or no adhesion to MHC[7,8] although overexpression in transfected fibroblasts has demonstrated binding by CD4 to class II and CD8 to class I[9,10]. The main physiological importance of these molecules is in signalling: when their contribution is blocked by antibodies, T cells require 100-fold higher concentrations of antigen to induce responsiveness[5,11–14].

Synergistic signalling by the association of the TCR with its co-receptor has been directly demonstrated for CD8 (ref. 15), which has been shown to bind to the membrane-proximal domain of the MHC class I molecule that is not polymorphic[15,16], whereas the TCR binds to the polymorphic membrane-distal domains[17]. The evidence on CD4 is less direct; but when CD4$^+$ helper cells form conjugates with antigen-presenting B cells, both the TCR and CD4 redistribute to the site of adhesion[14], and antibodies that induce the association of CD4 with the TCR also activate T cells[11,14]. The signalling function of CD4 and CD8 may depend on the association of their cytoplasmic segments with a lymphocyte-specific tyrosine kinase encoded by *lck* (ref. 18).

CD4 and CD8 may play a critical part in the differentiation of T-cell precursors in the thymus. Early in thymic ontogeny, immature T cells express both CD4 and CD8; coassociation of one or the other of these molecules with the T-cell receptor may signal to the thymocyte whether its TCR recognizes class I or class II MHC, and thereby regulate subsequent differentiation into CD8$^+$ cytotoxic or CD4$^+$ helper T-lymphocyte subsets[11,12].

Activation-dependent adhesion mechanisms

The definition of other cell-surface molecules essential for the

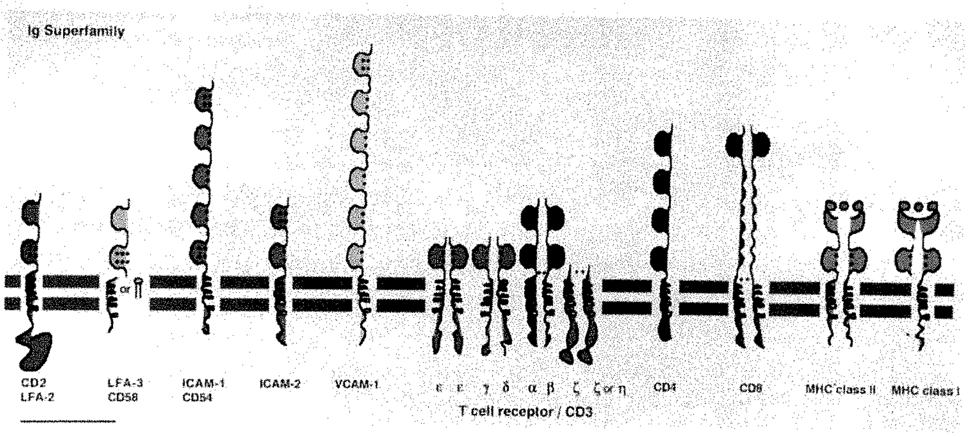


FIG. 1 Immunoglobulin superfamily adhesion receptors. Members of the superfamily share the immunoglobulin domain, composed of 90–100 amino acids arranged in a sandwich of two sheets of anti-parallel β-strands, which is usually stabilized by a disulphide bond at its centre[136,137]. The immunoglobulins and TCR, which are specialized for antigen recognition, are the only known members of this family with variable regions that undergo somatic diversification. The function of molecules of the superfamily in adhesion evolutionarily predates specialization for antigen recognition, which occurs only in vertebrates; immunoglobulin superfamily members are present in insects as nervous system adhesion molecules involved in axon guidance and fasciculation[138]. The immunoglobulin domain may have diversified and been adopted so widely in evolution because its stable disulphide-bonded β-strand structure is analogous to a car chassis on which many different styles of bodies and bumpers may be hung. These latter may be analogous to the loops connecting the β-strands, and also to the alternating residues in the β-strands that point outward away from the interior of the domain. An interesting feature of adhesion molecule structure is that, in contrast to immunoglobulins and MHC molecules, which have paired immunoglobulin domains, the domains of ICAM-1 (ref. 130) and NCAM (ref. 139) are unpaired. Immunoglobulin domains studied by X-ray crystallography are ellipsoids with a dimension of 4 nm parallel to the β-strands and 2.5 nm in the two perpendicular dimensions[17,137]. Electron micrographs of ICAM-1 show that it is a bent rod 18.7 nm long. This is only compatible with a model in which its five domains are unpaired, and arranged end-to-end at a slight angle to the β-strands. Overall size and shape of the immunoglobulin domains are shown to scale (bar, 10 nm), although schematized, see Fig. 8 for a more accurate scale drawing; dots denote disulphide-bonded cysteines. Dimensions are based on the following information: MHC class I molecule, X-ray crystallography[17]; MHC class II, approximation as MHC class I; TCR, approximation of $\alpha\beta$ heterodimer as an Fab fragment[140]; ICAM-1, electron microscopy[130]; ICAM-2 (ref. 29), CD2, LFA-3 (ref. 136), CD4 (ref. 13) and VCAM-1 (ref. 70) approximation to two, four or six unpaired domains as in ICAM-1; CD8, a disulphide-linked α–α or α–β dimer of one immunoglobulin domain, the length of the connecting or hinge-like peptide[13] of 40 or 65 amino acids for α or β, respectively, is arbitrary and is consistent with a spacing of 1.5–3.3 Å per residue[141]; note that the distance the connecting peptide is shown to extend in CD8 is similar to that which would be predicted for three of the four immunoglobulin domains in CD4, allowing the N-terminal domain of CD4 to occupy a position similar to that shown for the CD8 domain.

interaction of T cells with their targets has been focused principally on killer T-cell interactions, largely because of the simplicity of the killing assay[5,19-21]. Thus, three molecules have been identified by screening monoclonal antibodies for their ability to inhibit killing and were operationally termed lymphocyte function-related antigens: LFA-1, LFA-2 (CD2) and LFA-3 (Fig. 4*a*, *b*). These molecules are now known to account for the antigen-independent adhesion that is induced by prolonged antigenic stimulation of T cells *in vitro*[7,8,19,22,23] and presumably help localize activated T cells to sites of antigen accumulation in the lymph nodes *in vivo*[24].

LFA-1, which is a member of the integrin family (Fig. 2), is expressed on T lymphocytes; its counter-receptor on the target cell is ICAM-1 or ICAM-2 (for intercellular cell adhesion molecule), both members of the immunoglobulin family (Fig. 1)[19,21,23,25-29]. LFA-2 or CD2, another member of the immunoglobulin family, is expressed on the T lymphocyte: its counter-receptor on the target cell, LFA-3, is also a member of the immunoglobulin family[5,19,30,31] (Fig. 1). The LFA-1/ICAM and CD2/LFA-3 interactions can be shown by the effects on adhesion of monoclonal antibodies against the individual molecules to be independent; monoclonal antibodies to any of them, or to the TCR or CD8, can inhibit T-lymphocyte killing, showing that it is a highly complex process requiring cooperation between several different surface molecules[5,20,21,32]. Indeed, we shall see later that binding of the T-cell receptor can modulate

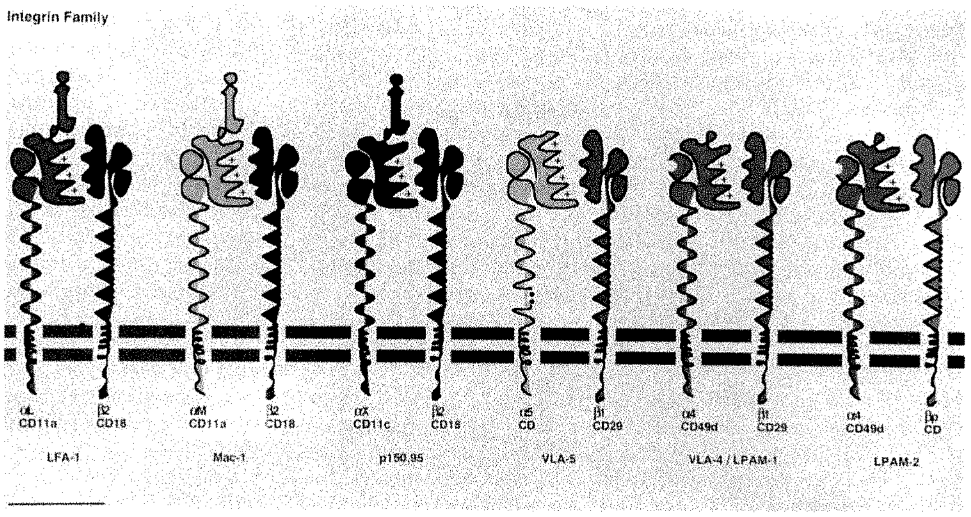


FIG. 2 Representative integrin family adhesion receptors. Integrins contain α- and β-subunits of approximately 1,100 and 750 amino acids, respectively, which are noncovalently associated. The α-subunits are 25–65% identical in amino-acid sequence and the β-subunits are 37–45% identical; the structural and functional similarities are so strong that integrins should be considered a protein family rather than a superfamily[21,65]. Although schematic, the overall size and shape of the integrins is shown to scale, based on electron microscopy of VLA-5 (ref. 142) and CD41/CD61 (ref. 143) (bar, 10 nm); the I domain is shown sticking out for emphasis but it may fold up with the rest of the globular head. The dots denote cysteine-rich regions in the β-subunit and a disulphide bridging a cleavage site in some α-subunits. Divalent cation binding sites are symbolized by '+'. Structures are cited in refs 21, 67 and Table I.

the affinity of LFA-1 for its counter-receptor. Adhesive interactions between CD2 and LFA-3 are also regulated by activation of T cells, although by a different mechanism.

Regulated adhesion by the CD2/LFA-3 interaction

The interaction between cells bearing CD2 and LFA-3 is finely poised, and is tipped toward adhesion by T-cell activation. This is reflected in 'rosetting' (Fig. 5), in which erythrocytes, which express LFA-3, adhere *in vitro* to activated T cells expressing CD2: resting T cells do not form rosettes with autologous erythrocytes.

The increase in adhesion between CD2 and LFA-3 on T-cell activation may be largely due to the regulation of the negative charge on the T-cell surface, which is mainly due to sialic acid[33]. Close cell-cell contact between circulating cells is opposed by the charge repulsion and by the decrease in entropy required for interdigitation of the surface glycocalyces[34]; these repulsive interactions seem to be reduced in activated T lymphocytes. The effect of activation on the CD2/LFA-3 interaction can be mimicked by the addition of positive charge through chemical derivatization of erythrocytes or removal of negative charge from erythrocytes or T cells by digestion of the glycocalyx[35,36]. Charge neutralization alters the morphology of the contact between T lymphocytes and erythrocytes, converting small islands of close contact surrounded by larger areas of greater intermembrane distance to a single large area of close contact[36]. Despite their larger surface area, T-cell blasts and thymocytes have fivefold less sialic acid per cell than resting T cells[37] and are less negatively charged[38]; this may be a primary factor determining whether the CD2/LFA-3 mechanism and other adhesion mechanisms are active or latent. In lymph nodes, the activated antigen-responsive lymphocytes that aggregate in germinal centres are greatly undersialylated, whereas areas containing B and T cells in rapid transit between blood and lymph are normally sialylated[39]. In the nervous system as well, cell interactions are regulated by sialylation; polysialylation of NCAM antagonizes its ability to promote adhesion[40]. Theoretical calculations[41] confirm that the attractive and repulsive energies in rosetting are of a similar order of magnitude and thus finely balanced, based on the affinity of the CD2/LFA-3 interaction of $10^6\ \mathrm{M}^{-1}$ (refs 30, 42, 43), adhesive receptor surface density and charge density[163].

CD2 may transduce a signal which augments or synergizes with signals from the TCR[5]. Certain pairs of antibodies against CD2, or combination of one such antibody with multimeric LFA-3, can stimulate T cells, but CD2/LFA-3 interaction alone has no effect[42,44]. Transfection of cells with CD2 and LFA-3 has confirmed early antibody inhibition results[19] showing CD2/LFA-3 interaction can contribute a 4- to 30-fold enhancement of the immune response[45,46]. The unusually basic, histidine- and proline-rich 120-amino-acid cytoplasmic region of CD2 is required for stimulation by pairs of monclonal antibodies[46,47]; in antigen-specific responses, however, truncation of the cytoplasmic domain of CD2 has given ambiguous results, leaving unclear the relative contributions of adhesion and signalling to enhancement of the immune response by CD2/LFA-3 interaction[45,46].

There are two isoforms of LFA-3, derived by differential messenger RNA splicing: one is anchored in the membrane by a glycophosphatidyl inositol tail, whereas the other has a classical transmembrane hydrophobic segment and a 12-amino-acid cytoplasmic segment[48-50] (Fig. 1). Both are fully active in mediating CD2-dependent adhesion and in promoting T-cell function[42,45,46,51]. The functional significance of the isoforms remains obscure, although it is intriguing that the neural cell adhesion molecule (NCAM) also has anchor isoforms[40,52].

ICAM-1 and ICAM-2 counter-receptors for LFA-1

LFA-1, although originally identified by monoclonal antibodies that inhibit T cell-mediated killing, is also required for a broad range of other leukocyte functions, including T-helper and B-lymphocyte responses, natural killing, antibody-dependent cytotoxicity mediated by monocytes and granulocytes, and adherence of leukocytes to endothelial cells, fibroblasts and epithelial cells[19,21]. A counter-receptor for LFA-1, ICAM-1, was identified using a simple assay called homotypic adhesion (Fig. 6*a*), in which homogeneous cell populations such as B- or T-cell lines adhere to one another to form multicellular clusters[19,53]. Aggregates form in this assay only if the lymphocytes have been stimulated with phorbol esters, and adhesion is completely inhibited by monoclonal antibodies against LFA-1 and is not observed with cell lines established from patients genetically deficient in LFA-1 (see below). LFA-1$^+$ cells can however coaggregate with LFA-1$^-$ cells[54]; and an LFA-1 counter-receptor, ICAM-1, was defined by immunizing mice with LFA-1$^-$ cells, and selecting monoclonal antibodies that would inhibit LFA-1-dependent homotypic adhesion[55]. ICAM-1 is a member of the immunoglobulin superfamily[27,28], with five immunoglobulin domains (Fig. 1).

In contrast to LFA-1, which is restricted to leukocytes, ICAM-1 can be expressed on a wide variety of cells and its induction in inflammation is an important means of regulating LFA-1/ICAM-1 interactions[21,53] and thereby presumably inflammatory responses. In the absence of an inflammatory response, ICAM-1 is expressed on only a few cell types[56]. Its importance has been demonstrated *in vitro* by blocking T-cell killing with antibody to ICAM-1 (ref. 57), and by transfection experiments in which fibroblasts expressing suboptimal levels of MHC molecules can be enabled to activate T-helper cells by cotransfection with the gene encoding ICAM-1[58]. This is consistent with its natural expression during the response to a specific antigen. ICAM-1 is expressed both on the follicular dendritic cells and on the

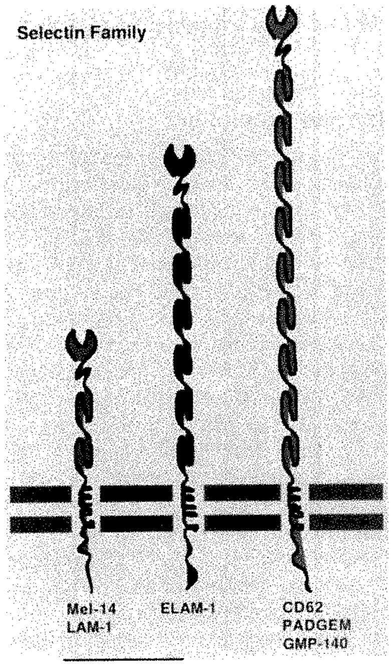


FIG. 3 Selectins. Selectins have an N-terminal domain of 117–120 amino acids which is homologous to a variety of Ca^{2+}-dependent animal lectins[144] including hepatic galactose receptors, soluble mannose-binding lectins, and invertebrate lectins, as well as to proteins known to bind ligands independently of carbohydrate, including the low-affinity receptor for IgE (CD23). After the N-terminal lectin domain is an EGF (epidermal growth factor) motif of 34–40 amino acids, and then short consensus repeat motifs of 62 amino acids, as found in many proteins involved in regulating complement activation. Structures are from refs 110, 112–115. Selectin domains are shown roughly to scale (bar, 10 nm): the short consensus repeats (SCR) extend 4.1 nm each, based on a length of 33 nm (ref. 145) for 8 SCR (ref. 146); EGF repeats extend about 2.3 nm (ref. 147), and the lectin-like N-terminal domain is modelled as a globular sphere[144].

activated B lymphocytes found in germinal centres[56], and may contribute through homotypic aggregation to formation of germinal centres.

Inflammatory mediators, including lipopolysaccharide, γ-interferon (γ-IFN), interleukin-1 (IL-1) and tumour necrosis factor (TNF) cause strong induction of ICAM-1 in a wide variety of tissues and greatly increase binding of lymphocytes and monocytes through their cell surface LFA-1 (refs 19, 21, 53, 59). Endothelial, fibroblastic and epithelial cells vary as to which cytokines are capable of inducing ICAM-1 expression, and the types of mediators released may therefore help regulate differing patterns of cell localization induced by inflammatory stimuli. Binding of leukocytes to endothelium is the first step in localization of circulating cells at an inflammatory site (Fig. 7*a*). *In vivo*, ICAM-1 induction accompanies T cell-mediated hypersensitivity reactions in the skin[60], and, after administration of γ-IFN and IL-1, appearance of ICAM-1 on endothelial cells correlates with sites of mononuclear cell infiltration[61]. ICAM-1 induction is largely regulated at the mRNA level[27,28]. Increased surface expression is first seen after 4 h, and is usually maximal by 24 h (ref. 53).

A second LFA-1 ligand, differing in tissue distribution from ICAM-1, was originally defined by the ability of antibodies against LFA-1, but not those against ICAM-1, to inhibit certain cell adhesion assays. On this basis, an ICAM-2 complementary DNA was isolated from an expression library by screening for binding of transfected cells, in the presence of monoclonal antibody against ICAM-1, to purified LFA-1 coated on Petri dishes[29]. ICAM-2 has two immunoglobulin-like domains, in contrast to ICAM-1 which has five (Fig. 1), and these are 35% identical to the two N-terminal domains of ICAM-1. ICAM-1 and ICAM-2 are much more similar to one another than to other members of the immunoglobulin superfamily, and thus represent a subfamily specialized to interact with LFA-1. Unlike ICAM-1, ICAM-2 is well expressed basally on endothelial cells and its mRNA is not increased by inflammatory mediators.

LFA-1 avidity and adhesion

The mechanisms discussed so far for regulating adhesive interactions operate on a relatively long timescale. Increased expression of ICAM-1 after cytokine induction is detectable *in vitro* or *in vivo* after 4-6 h, and is maximal by 9-24 h[21,53,61]. This time course is typical of regulation at the RNA level of surface adhesion receptor density, and seems to apply to CD2 and LFA-3 as well. Alteration of cell surface charge by changes in glycoprotein sialylation requires *de novo* glycoprotein biosynthesis[62] and glycoprotein turnover, which takes about 12-24 h. Yet adhesion by cytotoxic T cells can be regulated over a much shorter time scale; they can adhere to target cells, deliver a lethal hit, detach and engage with another target cell, with a cycle time as short as 1-5 min (ref. 63). Moreover, whereas cytotoxic cells stimulated *in vitro* show a general increase in adhesiveness, cells stimulated *in vivo* adhere only to those cells bearing the antigen to which they were primed[19,20]. This can be explained by the finding that crosslinking of the TCR on resting T lymphocytes transiently stimulates adhesiveness through LFA-1, allowing regulation of adhesion and detachment over a timescale of minutes[23].

This rapid modulation of adhesion is due to qualitative rather than quantitative changes in the cell-surface expression of adhesive molecules. Homotypic adhesion of leukocytes (Fig. 6*a*), which is dependent on LFA-1 and ICAM-1, is stimulated by treatment for 1 h with phorbol ester but is accompanied by no increase in LFA-1 or ICAM-1 surface expression[54,55]. By testing the binding of cells expressing LFA-1 and ICAM to plastic substrates coated with either purified ICAM-1 or purified LFA-1 (Fig. 6*b*), however, it can be shown that stimulating resting T lymphocytes with phorbol esters or crosslinking the TCR with monoclonal antibodies converts cellular LFA-1 from a low- to a high-avidity state, with no change in surface density[23]. Cellular ICAM, by contrast, is constitutively avid. These changes influence the ability of stimulated peripheral blood lymphocytes to form conjugates with target cells. Conjugate formation is inhibited completely by LFA-1 and only marginally by CD2 monoclonal antibodies, showing that LFA-1 is primarily responsible for regulating the avidity of cell-cell interactions. Avidity peaks 5-10 min after stimulation of the TCR and returns to resting values by 30 min. The transience of the high-avidity state provides a mechanism for regulating lymphocyte deadhesion.

Thus, in the absence of antigen, the equilibrium governing adherence favours free, mobile T lymphocytes and efficient immune surveillance. Contact of TCRs with cells bearing specific antigen generates intracellular signals that lead to the conversion of LFA-1 to a high-avidity state and regulates LFA-1/ICAM-1-dependent adhesion in an antigen-specific manner. LFA-1 is an adhesion servomotor controlled by the TCR.

The integrin family

LFA-1 is a member of the integrin family (Table 1), perhaps the most versatile of the adhesion molecule families. Each integrin molecule comprises an α- and a β-subunit (Fig. 2) and three subfamilies of integrins can be distinguished by their β subunits: these are known as the β1 (CD29), β2 (CD18) and β3 (CD61) integrins.

LFA-1 belongs to the β2 subfamily and is most closely related to two other integrins, Mac-1 and p150,95 with which it shares the β2 subunit[25] (Fig. 2). These three β2 integrins are also known as the leukocyte integrins because their expression is limited to white blood cells. Mac-1 and p150,95 are particularly important in adhesion of myeloid cells to other cells and to ligands that become insolubilized during activation of the complement and clotting cascades[21] (Table 1). The importance of the leukocyte integrins is illustrated in congenital leukocyte adhesion deficiency (LAD) in which they are deficient because of mutations in the common β2 subunit[21,64]. Patients have recurring infections, often fatal in childhood unless they are corrected by bone marrow transplantation. Neutrophils from these patients fail to orient and migrate in response to chemoattractants and are unable to bind to and cross the endothelium at sites of infection, so that pus fails to form. This is a most striking example of the role of adhesion molecules in leukocyte localization *in vivo*.

The β1 integrin subfamily includes receptors that bind to the extracellular matrix components fibronectin, laminin and collagen (Table 1) and they are expressed on many non-haematopoietic and leukocyte cell types. These receptors are likely to play a general part in tissue organization by binding to molecules in the extracellular matrix within many tissues and in the basement membranes found in muscle, the nervous system,

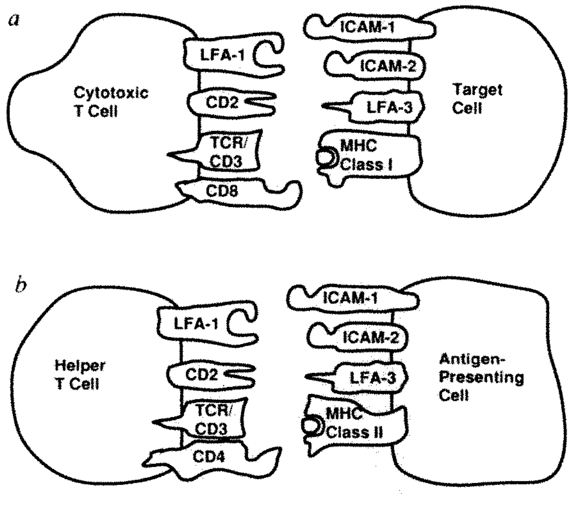


FIG. 4 Antigen-specific T-cell interactions.

TABLE 1 Integrin family of cell–cell and cell–matrix receptors

Subunits	Names	α-subunit* I	α-subunit* C	Ligands†	RGD role	Distribution Non-leukocyte‡	Distribution Leukocyte§	Ref.‖
$\alpha L\beta 2$	CD11a/CD18, LFA-1	+	–	ICAM-1,2	–		B, T, M, G	21
$\alpha M\beta 2$	CD11b/CD18, Mac-1, CR3	+	–	C3bi, FX, FB	+?		M, G	21
$\alpha X\beta 2$	CD11c/CD18, p150, 95	+	–	?	?		M, G	21
$\alpha 1\beta 1$	CD-/CD29, VLA-1	?	–	LM, CO	–	F, BM	B′, T′	67
$\alpha 2\beta 1$	CD49b/CD29, VLA-2, gpIaIIa, ECMRII	+	–	LM, CO	–	P, F, EN, EP	T′	154–157
$\alpha 3\beta 1$	CD-/CD29, VLA-3, ECMRI		+	FN, LM, CO	–	EP, F		155
$\alpha 4\beta 1$	CD49d/CD29, VLA-4, LPAM-1	–	!	FN, VCAM-1	–	NC, F	B, T, M, LGL	68, 69, 71, 74, 76
$\alpha 5\beta 1$	CD-/CD29, VLA-5, FNR, gpIc/IIa, ECMRVI	–	+	FN	+	F, EP, EN, P	Th, T	155
$\alpha 6\beta 1$	CD49f/CD29, VLA-6, gpIc/IIa		+	LM	–	P	T	94, 158
$\alpha 4\beta p$	CD49d/CD-, LPAM-2	–	!	?	?		T	82
$\alpha 6\beta 4$	CD49f/CD-, $\alpha E\beta 4$		+	?	–	E		77–79
$\alpha IIb\beta 3$	CD41/CD61, gpIIbIIIa	–	+	FB, FN, vWF, FB	+	P		95
$\alpha V\beta 3$	CD51/CD61, VNR	–	+	VN, FB, vWF, TSP	+	EN	B′, M	95, 159, 160
$\alpha V\beta 1$	CD51/CD29	–	+	FN	+	NC, F		161
$\alpha V\beta 5$	CD51/CD-, $\alpha V\beta S$	–	+	VN, FN	+	C, F, EP	M	80, 81, 160

*I, I domain; C +, cleavage to disulphide-linked heavy and light chains at amino acid 853–860; !, cleavage to nondisulphide-linked chains at roughly amino acid 573.

† LM, laminin; CO, collagen; FN, fibronectin, FB, fibrinogen; FX, factor X; VN, vitronectin; vWF, von Willebrand factor; TS, thrombospondin.

‡ EN, endothelial cells; EP, epithelial cells; F, fibroblasts or other connective tissue; NC, neural crest, melanocytes; P, platelets; C, carcinomas; BM, basement membrane-associated.

§ B, B lymphocytes; T, T lymphocytes; ′, activated lymphocytes only; Th, thymocytes; M, monocytes; G, granulocytes; LGL, large granular lymphocytes. Some data from Leukocyte Typing Database III (ref. 162) and IV.

‖ General refs 2, 21, 65–67.

and underlying the epithelium and endothelium[2,65,66]. The $\beta1$ family molecules have been designated VLA (very late activation) because two of them, VLA-1 and VLA-2, appear on lymphocytes 2–4 weeks after antigen stimulation *in vitro*[67]. In fact, some VLA molecules are basally expressed on leukocytes, and their expression on nonhaematopoetic cells does not require activation (Table 1). Induction of VLA-1, -2, -3 and -5 expression after leukocytes cross the endothelial barrier may be of great importance in controlling leukocyte localization in inflammation.

VLA-4 (CD49d/CD29) is an unusual $\beta1$ integrin expressed on resting lymphocytes, monocytes and neural crest-derived cells, and functions as both a matrix and cell receptor[67]. As a matrix receptor, it binds to an alternatively spliced domain of fibronectin distinct from the classical cell binding site recognized by VLA-5[68,69]. As a cell receptor, it binds to a molecule recently described as VCAM-1 or INCAM110 that is a member of the immunoglobulin superfamily[70–72]. This molecule is induced by inflammatory mediators on endothelium with kinetics similar to ICAM-1 and its interaction with VLA-4 provides an explanation for earlier evidence of a second lymphocyte–endothelium adhesion mechanism distinct from the LFA-1/ICAM interaction[59,73] (Fig. 7*b*). In congenital deficiency of the $\beta2$ integrins, which does not affect VLA-4, lymphocytes retain the ability to migrate across the endothelium at inflammatory sites[64]. This seems related to expression of VLA-4 by lymphocytes and not by neutrophils (Fig. 7*c*). Involvement of VLA-4 in T cell-mediated killing[74] and in homotypic adhesion[75] suggests some functional redundancy with LFA-1. VLA-4 also helps mediate lymphocyte recirculation[76], as described below.

The complexity of the integrin family has recently been increased by the discovery of novel β-subunits that can associate with the $\alpha4$, $\alpha6$ and αV subunits alternatively to the previously described $\beta1$ and $\beta3$ subunits[77–82] (Table 2). Both α- and β-subunits affect ligand specificity. Their combinatorial use confers considerable diversity in ligand recognition, and differences in transmembrane and cytoplasmic domains may also help regulate communication between the inside and outside of the cell.

Structure and regulation of integrins

The structural domains of integrins (Fig. 2) have been correlated with ligand binding by crosslinking to peptides containing the sequence Arg-Gly-Asp (RGD in the single-letter amino-acid code), a ligand recognition motif for several but not all integrins (Table 1). On the $\beta3$ subunit, ligand peptides are crosslinked within residues 109–171 (ref. 83). This is the most highly conserved region among the $\beta1$, $\beta2$ and $\beta3$ subunits, and in LAD single amino-acid substitutions in this region of $\beta2$ prevent association with the α-subunit (ref. 84); thus close association of the α-subunit with this region of the β-subunit may form a ligand-binding pocket. Integrin α-subunits have three or four tandem repeats of a putative divalent cation-binding motif (Fig. 2), and require Ca^{2+} or Mg^{2+} for function[21]. LFA-1 α-subunit has three such repeats and binds Mg^{2+}; this correlates with the requirement for Mg^{2+} in T-cell adhesion and in binding of purified LFA-1 to purified ICAM-1 (ref. 23). The divalent metal-binding motif in integrins has only five of six predicted metal coordination sites. It has been proposed that in integrin binding to RGD-containing ligands, the Asp residue (D) in RGD binds to the metal held in the divalent cation-binding pocket of the α-subunit, forming a sixth coordination site[85]. Consistent with this, a ligand is crosslinked to amino acids 294–314 of the α-subunit of $\alpha IIb\beta 3$, which define the second divalent cation-binding site[86].

Further integrin domains may be involved in ligand binding. All three leukocyte integrin α-subunits and the VLA $\alpha2$ subunit have a domain of 200 amino acids not present in other integrin α-subunits, and hence termed the 'inserted' or I domain. The I domains are homologous to ligand-binding repeats in von Willebrand factor and other proteins, and may confer modes of ligand recognition in addition to those shared by all integrins[21]. Cysteines are notably few in the putative ligand binding regions of the α- and β-subunits, permitting conformational changes that regulate ligand binding.

Interactions of integrins with the cytoskeleton may be regulated by binding to ligands, and conversely, may help regulate ligand binding, thus mediating a bidirectional dialogue across the membrane. Several of the integrins can localize near to focal contacts, areas where the cell membrane is closely opposed to the extracellular matrix substrate and where actin bundles terminate, surrounded by a ring of vinculin and talin[87]. Talin seems to interact with the cytoplasmic domain of $\alpha5\beta1$ (ref. 88). Talin redistributes with LFA-1 to sites of antigen-specific adhesion and cocaps with LFA-1 after stimulation with phorbol ester[14]; talin association may be a widespread feature of integrins. It is intriguing that redistribution of LFA-1 and talin is highly sensitive to low antigen concentrations and may correlate with the high-avidity state of LFA-1[89].

Like many receptors, integrins transduce information from the outside to the inside of the cell. The growth and differentiation of many connective tissue and nervous system cells is affected by their substrates, largely through integrins[2,65,66]. Examples in the immune system include regulation of T-cell proliferation by LFA-1 (refs 90–92), VLA-5 (ref. 93), VLA-4 and VLA-6 (ref. 92).

Integrins are novel receptors with respect to the type of inside-out signalling described for LFA-1, in which signals from the cytosol are transduced across the membrane to generate changes in extracellular functions such as adhesion. The avidity of other integrins besides LFA-1 seems to be regulated. On lymphocytes, the avidity of the $\beta 1$ integrins VLA-4 and VLA-5 for fibronectin and VLA-6 for laminin is increased analogously to LFA-1 after TCR crosslinking[94]. On unactivated platelets, the integrin gpIIbIIIa does not bind fibrinogen, but on activation binds soluble fibrinogen with a dissociation constant K_d of 29-45 nM (ref. 95). The high-avidity state of gpIIbIIIa seems to be permanent rather than transient. The mechanism of avidity regulation is unclear, but the ability of antibodies to detect conformational changes in LFA-1 (ref. 96) and in gpIIbIIIa (ref. 95) in sites distinct from the ligand-binding site suggests conformational changes in the ligand binding site may also occur. On stimulation with chemoattractants, neutrophils transiently adhere to other neutrophils, endothelial cells and to C3bi-coated cells and there is some evidence that this may be due to a transient change in avidity of Mac-1 (refs 97-99). The cytoplasmic domains of integrins, perhaps by interaction with other cellular proteins, may signal the changes in the extracellular domains that regulate avidity. The LFA-1 β-subunit (integrin $\beta 2$) cytoplasmic domain is required for functional activity in binding to ICAM-1, as shown by transfection of patient cell lines that genetically lack the β-subunit with intact and truncated β-subunit cDNAs (ref. 100, and M. L. Hibbs, H. Xu, S. A. Stacker and T.A.S., unpublished data).

Lymphocyte education

Lymphocytes newly emigrated from the thymus are considered 'naive', and remain so until they encounter and are stimulated by specific antigen. They then become longer-lived 'memory' lymphocytes. As discussed above, transient alterations in adhesion mechanisms lasting for minutes to days accompany lymphocyte activation. But permanent alterations in surface density also occur, as a result of the transition from the naive to memory phenotype. Shortly after antigen stimulation, naive T lymphocytes of both CD4$^+$ and CD8$^+$ subsets acquire increased levels of a cohort of surface molecules including the adhesion receptors CD2, LFA-1 and VLA family members[94,101,102] (Table 2). Increased expression of these surface molecules persists after the stimulated lymphocytes have reverted to the resting state, probably lasting for the life of the memory cell. The changes in surface phenotype of memory T cells may have important consequences for their localization because they occupy distinct microenvironments within lymphoid organs[103] and have different recirculation routes[104].

TABLE 2 Conversion of naive to memory T lymphocytes alters surface molecule phenotype*

Molecule	Difference in expression (-fold increase)
CD2	2.8
LFA-1	2.4
LFA-3	− to +
CD49d (VLA-4)	2.7
(VLA-5)	3.6
CD49f (VLA-6)	3.4
CD29 (VLA-β, 4B4)	3.7
CD44 (Hermes Pgp-1)	2.1
CD45RO (UCHL1)	29
CD45 RA (2H4)	+ to −
CD4	1.0
CD8	1.0
TCR (CD3)	1.0

* Modified from refs 94, 102.

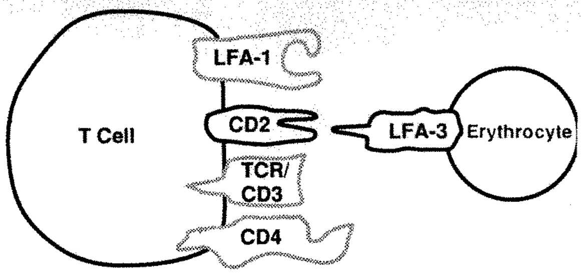


FIG. 5 T-cell rosetting with erythrocytes.

Naive and memory T-cell subsets differ in lymphokine secretion, in some functional assays[101,102,105,106], and in other important respects. Memory T cells seem to be more sensitive to antigen, because they are responsive to stimulation by much lower concentrations of monoclonal antibody against TCR, although they have quantities of TCR, CD8 and CD4 identical to naive T cells[107]. Increased expression of the LFA-1 and CD2 molecules should also enhance their sensitivity to antigen by facilitating interactions with antigen-presenting cells.

Lymphocyte recirculation receptors

Patrolling the body in search of foreign antigen, lymphocytes leave the blood, migrate through lymphoid organs and other tissues, and enter the lymphatics, whence they return to the blood through the thoracic duct. The peripheral lymph nodes draining the skin and the Peyer's patch and gut-associated lymph nodes draining mucosal surfaces differ in the types of antigens to which lymphocytes are exposed. Lymphocytes from adult animals differ from those in newborns in that, when collected from specific lymph nodes, they show a twofold preference for recirculation to lymph nodes of the type from which they came[24,108]. This suggests that priming by specific antigen may alter surface phenotype to enable selective recirculation to the type of secondary lymphoid organ where specific antigen was first encountered.

Lymphocytes in the blood enter lymph nodes by binding to specialized 'high' endothelial cells. Lymphocyte suspensions overlaid on sections of lymph nodes bind to these specialized venules, and 'recirculation' or 'homing' receptors on lymphocytes have been defined by monoclonal antibodies that block binding to the high endothelial cells of specific types of lymph nodes[24,108,109]. Molecules, termed 'addressins', selectively expressed on specialized high endothelium in different types of lymph nodes, are good candidates for the homing receptor ligands[24,108,109]. Binding and immigration to lymph nodes may be a cooperative process involving several receptors on the lymphocyte and counter-receptors on the endothelium, analogous to antigen-specific interactions (Fig. 4) because CD44, LFA-1, VLA-4 and Mel-14/LAM-1 on the lymphocyte have all been implicated in binding to high endothelial venules (HEV). The more interesting candidates for specific receptors are Mel-14/LAM-1 as a peripheral lymph node receptor, and the VLA-4 α-subunit associated with either of two integrin β-subunits as a Peyer's patch receptor[24,82,108-110]. But the function of these molecules in adhesion hardly seems limited to lymphocyte recirculation, as discussed above for VLA-4.

Alternatively to emigration through HEV, lymphocytes may emigrate through endothelium within a tissue such as skin, and enter a lymph node through the afferent lymph. Memory T lymphocytes almost exclusively emigrate from blood through tissue endothelium, whereas naive lymphocytes emigrate through HEV (ref. 104). It is thus possible that the endothelia of skin and mucosa may differ in expression of ligands that enable selective recirculation through these tissues. Congruent with this idea, the candidate homing receptors CD44 and VLA-4 show increased expression on the memory T-lymphocyte subset (Table 2) and LAM-1 (Leu8/TQ1) is preferentially expressed on a distinctive but overlapping memory lymphocyte subset[111]. Differences of several fold in surface density of these receptors may be adequate to give rise to selectivity in recirculation.

The role of selectins

The Mel-14/LAM-1 molecule is a representative of a novel class of molecules termed 'selectins', with an N-terminal lectin domain and diverse roles in adhesion[109,110,112-115] (Fig. 3). The finding of the lectin-like domain in Mel-14/LAM-1 correlates with the Ca^{2+} requirement for lymphocyte binding to peripheral lymph node endothelium and evidence that the counter-receptor is carbohydrate-like[108-110,113]. The number of short consensus repeats, which varies from two to nine in the three different selectins discovered so far, may serve to position their N-terminal lectin-like putative binding sites at varying distances from the plasma membrane (Fig. 3).

All three selectins help regulate leukocyte binding to endothelium at inflammatory sites (Fig. 7*c*). The selectin Mel-14 not only functions as a lymphocyte recirculation receptor, but also contributes to neutrophil emigration at inflammatory sites[24,116]. After stimulation of neutrophils with chemoattractants, Mel-14/LAM-1 is rapidly shed from the cell surface[117]. The endothelial leukocyte adhesion molecule, ELAM-1, is a selectin that is transiently expressed on endothelial cells 2-8 h after stimulation with IL-1 and other inflammatory agents, and mediates a neutrophil adhesion pathway distinct from that mediated by ICAMs and leukocyte integrins[112,118]. The neutrophil chemoattractant IL-8, which is secreted by activated endothelial cells, acts on neutrophils to inhibit binding to ELAM-1 (ref. 119). The proteolytic release of Mel-14 from the cell surface upon neutrophil activation, and the similar inactivation of the ELAM-1 counter-structure on the neutrophil, suggests that Mel-14 and ELAM-1 function in an early step in neutrophil binding to the endothelium, before transendothelial migration. This contrasts with integrins, which are increased on the neutrophil surface by mobilization from granule compartments within minutes after stimulation of neutrophils with chemoattractants, and then remain permanently upregulated[64,117]. Although both selectins and integrins can regulate neutrophil adhesion to endothelium, when selectins mediate adhesion, integrins are still required for the subsequent event of transendothelial migration[120,121] (Fig. 7*a*). This may explain why congenital deficiency of the leukocyte integrins so completely inhibits neutrophil emigration from the blood[64].

A third selectin called PADGEM, GMP-140 or CD62 is stored in α-granules of platelets and Weibel-Palade bodies of endothelial cells, and is rapidly mobilized to the surface of these cells after stimulation by products of the clotting cascade such as thrombin, where it mediates adhesion of neutrophils and monocytes[115,122]. Thus, selectins function in a wide range of cell interactions in the vasculature and are expressed both on leukocytes and endothelial cells.

Virus receptors

Several cell adhesion receptors are subverted as virus receptors[123]. The human immunodeficiency virus (HIV), which causes AIDS, binds to CD4. Although rhinoviruses, which cause common colds, have evolved more than 100 non-crossreactive antigenic variants in an attempt to evade the immune response, 90% of them bind to the same receptor, ICAM-1.

The use of cell-adhesion receptors by viruses may be more than coincidental. First, virus-cell and cell-cell adhesion are in principle very similar. Both require accessible cognate sites on the receptor, both involve multivalent interactions and both may be facilitated by receptor redistribution to the site of adhesion. Second, binding to molecules that participate in the immune response has important consequences for the host-virus relationship. Human immunodeficiency virus (HIV) infects and kills or renders anergic $CD4^+$ T-helper cells, and downregulates CD4 expression[124]. Binding of HIV or its shed surface glycoprotein gp120 may transduce signals through CD4 which interfere with normal responsiveness[125]. HIV can spread by fusion of infected cells with uninfected cells to form syncytia. This requires CD4 and gp120 but also LFA-1 (ref. 126). Rhinoviruses, by contrast, rather than thwarting the immune response, use it to their own ends. Mucous secretions and sneezing induced by the immune response facilitate infection of other individuals. If ICAM-1 has a signal transducing function on antigen-presenting cells, this may be mimicked by the virus and may serve its ends by stimulating production of cytokines which increase nasal secretions carrying the virus.

In view of the rationales discussed above, it is of considerable interest that viruses have evolved to bind to the same regions of CD4 and ICAM-1 as do their cell adhesion counter-structures. HIV binds to the first and part of the second immunoglobulin-like domains of CD4 (refs 127-129); the same overall region of CD4 binds to MHC class II molecules[129]. LFA-1 and rhinoviruses bind to overlapping but distinct regions of the first immunoglobulin-like domain of ICAM-1 (ref. 130). The binding site for ICAM-1 is hypothesized to be in a 'canyon' on the rhinovirus surface which is too narrow to admit an antibody[164], consisting of paired immunoglobulin domains, but is of the right dimensions to fit unpaired domains as exist in ICAM-1 (ref. 130).

Close encounters at the membrane

We are just beginning to understand adhesion on a molecular scale (Fig. 8). The size and shape of several immune system adhesion molecules are known by electron microscopy or X-ray crystallography, and others may be modelled from the structure of homologous molecules or constituent domains (Figs 1, 2 and 3 legends). The binding sites for several of the adhesion receptors and their counter-receptors are known (Fig. 8 legend), allowing prediction of how close together two cell membranes, or a cell membrane and the extracellular matrix, would have to come to allow interaction. A model of the distances over which adhesion receptors would mediate cellular interactions can then be constructed (Fig. 8). This model can be compared with measurements of distances made in micrographic studies[131,165]. Furthermore, the sizes of two major components of the leukocyte glycocalyx that bear most of the cell-surface sialic acid[132], CD43

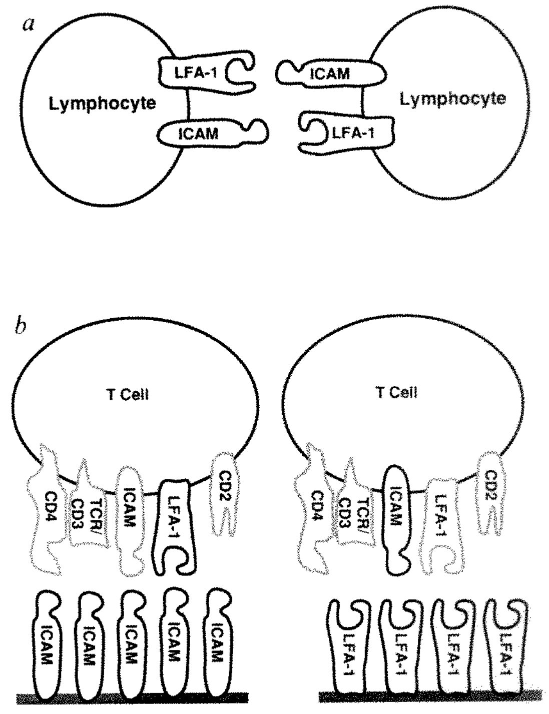


FIG. 6 Homotypic adhesion (*a*) and adhesion to purified molecules in artificial membranes (*b*).

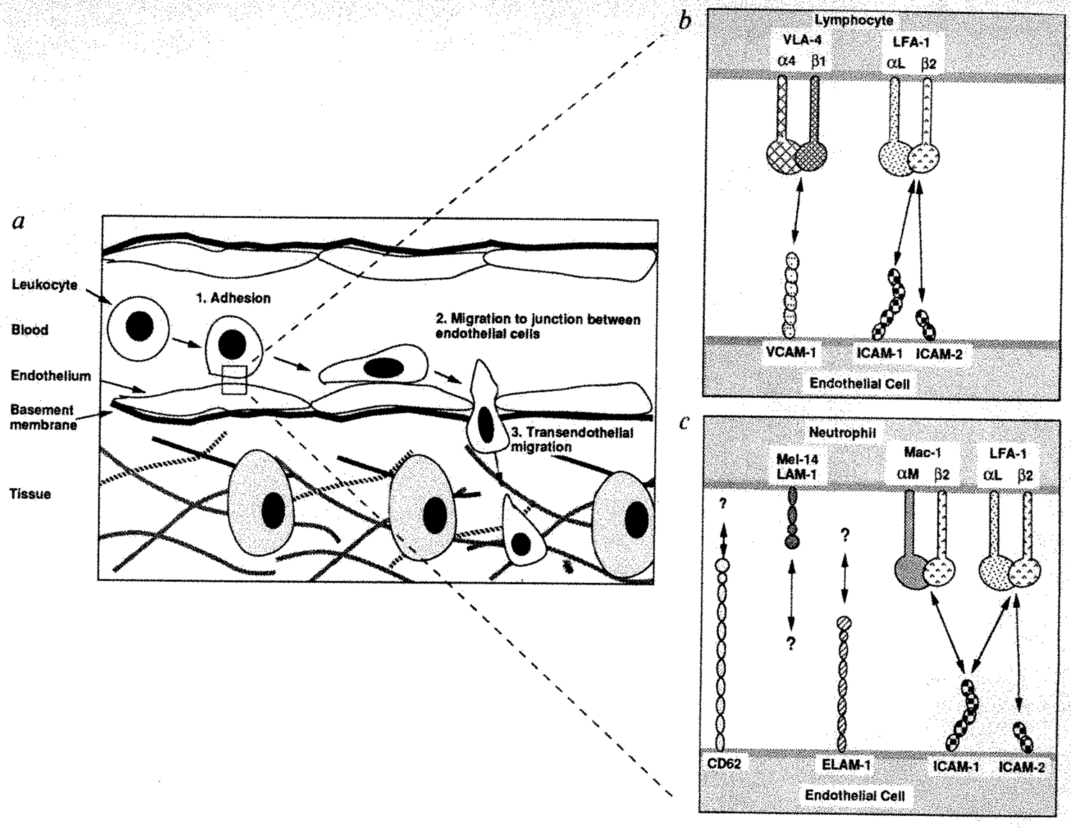


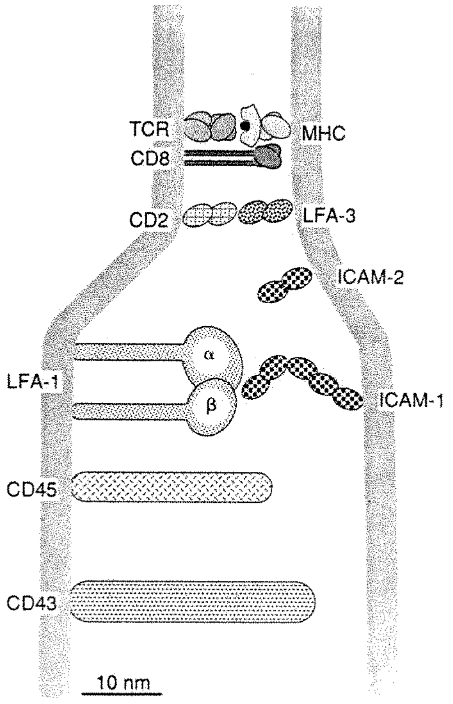
FIG. 7 Leukocyte extravasation (*a*), and mechanisms for adhesion of lymphocytes (*b*) and neutrophils (*c*) to endothelium.

and CD45, are included in Fig. 8 as a reminder of the scale of the glycocalyx, which will oppose cell–cell adhesion due to negative-charge repulsion and the loss of entropy involved in compressing or interdigitating the glycocalyces of two contacting cells[34].

The model (Fig. 8) predicts two classes of adhesion receptor interactions that differ significantly in the distance between the plasma membranes of the two closely apposed cells. The TCR–MHC and CD2/LFA-3 interactions are predicted to occur within an intermembrane distance of ~13 nm or less. This is compatible with electron microscopic measurements of intermembrane distances of 7.5 nm for point contacts of microvilli (0.2-μm wide) and 17 nm for broad areas several μm wide of membrane apposition in interactions of mitogen-stimulated T cells with target cells[165].

By contrast, the interactions of LFA-1 with ICAM-2 and ICAM-1 are predicted to occur within an intermembrane distance roughly equal to or less than 27 or 36 nm, respectively (Fig. 8). There may be important qualitative differences between these two types of membrane contacts, and they could occur in different membrane domains within the zone of adhesion between two cells. Very close contact mediated by the TCR–

FIG. 8 Scale models of interacting adhesion receptors. Immunoglobulin superfamily members and integrins are drawn to scale (Figs 1, 2 legends). Glycocalyx components: CD43, the 235-residue extracellular domain[148,149] with one in five *O*-linked amino acids[132] is modelled as 235/420 the length of glycocalicin (CD42b), which has 420 amino acids with a similar *O*-linked carbohydrate content[150,151]; CD45 is based on electron micrographs[152]. The lipid bilayer is drawn to scale as 4–5-nm thick; protein transmembrane and cytoplasmic domains are omitted. The presence of hinge or connecting regions between globular domains and the membrane-spanning domain introduces uncertainty into how far some of these molecules extend from the membrane, because an extended polypeptide would extend much farther than globular regions with the same number of amino acids. But connecting regions are absent or short except for CD8, and are already included in the lengths determined by electron microscopy for integrins and CD45. Carbohydrate side chains are not shown; the sizes of molecules based on electron microscopy would take into account both protein and carbohydrate. The MHC class I peptide-binding site is assumed to interact with the complementarity-determining region of the TCR[140]. The binding site in MHC class I for CD8 is in the membrane proximal $\alpha 3$ domain[15,16]; the binding site for MHC is assumed to be in the CD8 immunoglobulin domain, analogously to the MHC binding site in CD4 (ref. 129). ICAM-1 binds to LFA-1 through its N-terminal immunoglobulin domain[130]; the binding site in ICAM-2 is modelled in its homologous N-terminal domain. The ICAM binding site in LFA-1 is assumed to be in the membrane-distal globular domain of integrins as predicted[142]; the exact location within this domain will not affect overall conclusions. The binding site for LFA-3 in CD2 is in the N-terminal immunoglobulin domain[153], and the binding site in LFA-3 is assumed to be in a homologous position.



MHC and CD2/LFA-3 interaction would be expected to require extensive interdigitation of the membrane glycocalyces of the two adhering cells, hinder the lateral mobility of surface glycoproteins, and either exclude major glycocalyx components such as CD43 and CD45 from the zone altogether or require that they assume a less extended conformation or change their orientation to the membrane. Because the cytoplasmic domain of CD45 is a tyrosine phosphatase that acts on the tyrosine kinase associated with CD4 and CD8 (ref. 133), such considerations could affect signalling.

The longer range contacts mediated by the LFA-1 interaction with ICAM-1 and ICAM-2 are predicted to involve much less glycocalyx interdigitation. This may be compatible with lateral movement of one membrane surface relative to the other, without significant frictional drag imposed by interlocking of the apposed glycocalyces. Migrating cells do not possess focal contacts, which have a cell to substrate distance of 10-15 nm, but instead have close contacts with a cell to substrate distance of ~30 nm (ref. 131), in good agreement with the distance predicted for LFA-1/ICAM interaction, and analogously, for integrin-matrix interaction. There is now good evidence that lipids[134] and membrane proteins[135] are drawn forward by extension of the leading edge of the cell. Although evidence is best for the dorsal surface, results suggest that the ventral surface of the membrane also translates forward with respect to the substrate. LFA-1 and other integrins could serve as transiently fixed points in the membrane that connect the cytoskeleton to the extracellular environment, with a cell-cell or cell-substrate distance that allows membrane lipid and other proteins on the same cell surface to flow laterally in a tide drawn by membrane extension in another area of the cell. Localized changes in integrin avidity, with high avidity at the leading edge of the cell and low avidity at the trailing edge may help regulate and drive cell migration[23], in conjunction with tension generated by the cytoskeleton. These two mechanisms for regulating cell migration may be coordinated by interactions between the cytoplasmic domains of integrins and the cytoskeleton.

Concluding remarks

I have emphasized the dynamic role of lymphocyte adhesion receptors in regulating lymphocyte antigen-specific interactions, localization in lymphoid and nonlymphoid organs, and in bidirectionally transmitting information which affects cellular differentiation and responsiveness and interaction with the environment. Adhesion receptors modulate interactions on different temporal scales and at different distances from the cell surface. There are important interactions between antigen receptors and adhesion molecules involving signalling pathways and interactions with gene expression. Further studies on three-dimensional structure and interactions with signalling pathways and the cytoskeleton promise to provide exciting insights into the mechanism of function of adhesion receptors. The role of these molecules *in vivo* in guiding cell interactions and localization in the complex microarchitecture of lymphoid organs, as well as in immune responses in other tissues, is another area that promises to yield rich insights. □

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ARTICLES

A 1,400-year tree-ring record of summer temperatures in Fennoscandia

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Tree-ring data have been used to reconstruct the mean summer (April–August) temperature of northern Fennoscandia for each year from AD 500 to the present. Summer temperatures have fluctuated markedly on annual, decadal and century timescales. There is little evidence for the existence of a Medieval Warm Epoch, and the Little Ice Age seems to be confined to the relatively short period between 1570 and 1650. This challenges the popular idea that these events were the major climate excursions of the first millennium, occurring synchronously throughout Europe in all seasons. An analysis of past warming trends suggests that any summer warming induced by greenhouse gases may not be detectable in this region until after 2030.

THE collection of living and remnant Scots pine (*Pinus sylvestris* L.) from the Torneträsk region in northern Sweden has been reported previously[1]. This material has yielded absolutely dated series of raw mean ring widths[2] and maximum latewood densities[3] extending unbroken from the present back to AD 436 and AD 443, respectively. At high latitudes, both of these variables are known to correlate well with climate data (specifically, summer temperatures[4–7]), but only qualitative inferences based on these particular long ring-width and density chronologies have been made to date[3,8]. Here we describe the reprocessing of the raw ring-width and density data to produce new chronologies and how they have been used to derive yearly estimates of mean Fennoscandian temperatures for a statistically defined 'summer' (April–August) season, reaching back to AD 500. These are the longest annually resolved climate reconstructions from tree rings yet published.

Torneträsk tree-ring chronologies

Standardization in dendroclimatology is a method for removing variability in tree-ring series that is not related to climate. It involves removing part of the low-frequency variability from the series of raw measurements[9]. The rationale behind this is a need to account for differences in the general growth rate or vitality of individual trees and to remove trends in individual tree-ring series that arise as a consequence of non-climate-

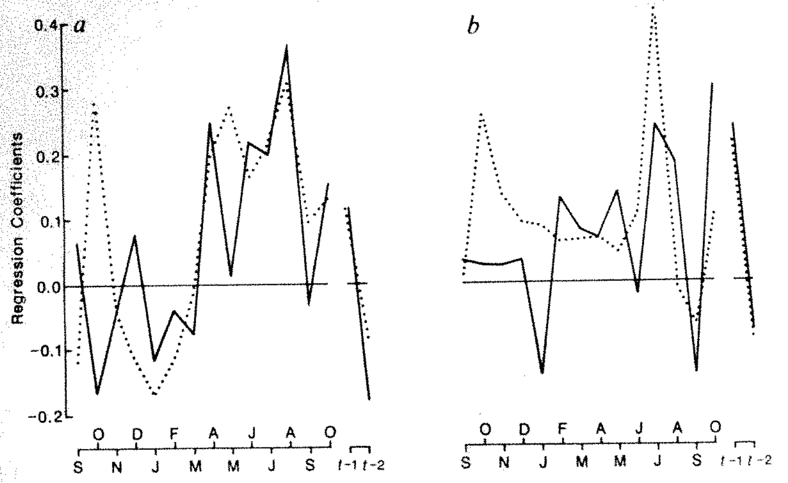


FIG. 1 Patterns of growth response (*a*, maximum latewood density; *b*, total ring width) in Torneträsk pine to intra-annual temperature forcing for the periods 1876–1925 (dotted line) and 1926–1975 (solid line), derived using multiple regression. The predictors include 14 monthly temperature series for the successive months from September of the year preceding ring formation, through to October immediately following the growing season. Two other variables, the relevant ring width or maximum latewood density for the two preceding years ($t-1$ and $t-2$) are also included to quantify the strengths of any biological preconditioning of growth in one summer by conditions in previous seasons.

related growth processes such as tree ageing or forest-stand development. Various deterministic, digital filtering and time-series approaches may be used[10]. Just what fraction of low-frequency variability is lost depends on the method applied. As a general rule, if efficient methods are used, the longer the constituent series, the longer is the timescale of potential climate variability that can be reconstructed from the mean standardized chronology.

The 65 ring-width and 65 maximum-density series that make up the chronologies produced here range in length from 92 to 612 years with a mean of 290 years (27 series are more than 300 years long). We have standardized the constituent data series using smoothing splines with a frequency-response cutoff set at two-thirds of the length of each series[10]. For example, in a 300-yr series, most of the variance above 200 years would be lost. The mean length of the splines fitted to all of the series is 194 years, but a number are long enough to preserve significant variance on timescales up to ~300 years. Although this standardization procedure effectively precludes any possibility of reconstructing climate variability on timescales much over 300 years, it is capable of preserving much of the variability on timescales approaching this.

The statistical reliability of a chronology is a function of the common growth signal (measured as the mean inter-series correlation coefficient) and the number of series (or replication depth)[11]. The best-replicated sections of the ring-width and density chronologies (the data from the nineteenth and twentieth centuries), explain over 90% of the variance of a hypothetical infinitely replicated chronology. This figure remains above 77% back to about 550 and is only marginally worse before this. The chronologies may therefore be considered statistically reliable and homogeneous time series over virtually their whole lengths.

Temperature effects

To define an optimum season for reconstruction and to help suggest the form of linear regression equation with which to model it, we carried out temperature-response function analysis for both the ring-width and densitometric chronologies[9]. This involves regressing principal components of the monthly temperature and previous growth data on the annual tree growth indices, to derive a set of regression coefficients that indicate the direction and relative strength of the temperature forcing on tree growth. The temperature data used were area averages formed by merging gridded data from 65° and 70° N at 10°, 20° and 30° E, producing mean monthly anomalies with respect to a base period of 1951-70[12]. The resulting series is considered homogeneous back to 1876.

Response functions were calculated and verified using data from 1876-1975, split into equal halves (see Fig. 1 and Table 1). Similar patterns of response are apparent over the two subperiods, although the similarity and strength of the response is somewhat greater in the case of the densitometric data. The largest regression coefficients show that maximum density is apparently enhanced by warm conditions during all of the months from April to August. The same is generally true for ring widths, except that the regression coefficients for April, May and June are noticeably smaller than those for density and the biggest width response is in July and August. The July temperature coefficient is the largest. The importance of July temperature in the growth of high-latitude pines is well documented[6,13,14].

These results suggest that April–August mean 'summer' temperatures may be usefully reconstructed using these tree-ring data. The relatively large coefficient on the previous year's ring width in the response function, which reflects the significant one-year-lag autocorrelation (r_1) in the ring-width series (ring width $r_1 = 0.38$, density $r_1 = 0.15$), means that a lagged ring-width variable must be included in the regression model for climate reconstruction. We therefore used a regression equation of the form

$$T_t = b_1\,\mathrm{TRW}_t + b_2\,\mathrm{TRW}_{t+1} + b_3\,\mathrm{MXD}_t + b_4\,\mathrm{MXD}_{t+1} \qquad (1)$$

where T_t is the mean April–August temperature of northern Fennoscandia in year t; TRW_t and TRW_{t+1} are the ring-width data in the years t and $t+1$ respectively and MXD_t and MXD_{t+1} are the equivalent maximum latewood densities.

Statistical reconstruction equation

Having established the potential for reconstructing the 5-month summer season (April–August) and chosen the form of regression model (equation (1)) we undertook a cross-calibration/verification exercise to test the general form of regression equation to be used for the reconstruction back to 500. The mean summer temperature series was divided into two 50-year periods 1876-1925 and 1926-1975. Equation (1) was then fitted over one period using a principal-component regression technique[15] and the derived coefficients applied to the tree-growth data over the other period to give estimates of temperature which could be compared with the observed climate data (see Table 2). This process was then repeated with the periods reversed. The early- and late-calibrated regression equations are very similar with a large positive weight on current-year maximum density. The results of the verification tests show that 44–49% of the independent temperature variance is recovered in the estimated data. All of the verification tests give results that are comparable to the best tree-ring-based climate reconstructions made to date.

Having satisfied ourselves of the validity of our general regression model we re-calibrated the reconstruction equation using all 100 years of climate data so as to maximize the timescale of variability against which the final regression equation could be fitted. The recalibrated regression coefficients (Table 2) are

TABLE 1 Response function analyses on Torneträsk chronologies

	Ring width			Maximum latewood density		
Calibration period	1876–1925	1926–1975	1876–1975	1876–1925	1926–1975	1876–1975
Verification period	1926–1975	1876–1925	—	1926–1975	1876–1925	—
Calibration						
R^2	0.49	0.32	0.51	0.66	0.58	0.56
R^2_{adj}	0.42	0.24	0.48	0.59	0.50	0.51
Verification						
r^2	0.31	0.29	—	0.37	0.44	—
RE	0.54	0.61	—	0.40	0.53	—
CE	0.09	0.13	—	0.25	0.44	—
PMt	5.3	3.6	—	3.3	4.1	—
First difference sign						
correct	32	27	—	35	31	—
incorrect	15	20	—	11	16	—
Significant regression coefficients ($P=0.05$) and their signs						
	Oct. ($t-1$) +	Feb. ($t-1$) +	Oct. ($t-1$) +	Oct. ($t-1$) –	Oct. ($t-1$) –	Jan. (t) –
	Nov. ($t-1$) +	Apr. (t) +	Feb. (t) +	Jan. (t) –	Jan. (t) –	Apr. +
	Jan. (t) +	July +	Mar. –	Apr. +	Apr. +	May +
	Mar. –	Oct. +	May +	May +	June +	June +
	Apr. +		June +	July +	July +	July +
	June +		July +	Aug. +	Aug. +	Aug. +
	July +		Aug. +	Oct. +	Oct. +	Density ($t-2$) +
			Oct. +			
	Ring width ($t-1$) +		Ring width ($t-1$) +			

R^2, square of the correlation coefficient calculated between actual and the estimated data; R^2_{adj} is the R^2 value adjusted to take account of the effective number of predictors; r^2 is the square of the actual/estimated correlation over the verification period; RE is the reduction of error[9]; CE of the coefficient of efficiency[7]; PMt is the t value derived using the product mean test and first difference sign is a test for comparing the signs of the first differences for the estimated and actual data[9].

essentially the same as those for the two subperiods. The three possible reconstructions (based on the early, late and overall periods) have ≥95% variance in common over the period 500–1980. Supporting evidence for the reliability of our temperature estimates comes from comparisons with fragmentary instrumental temperature records[16]. These exist for a number of sites in northern Fennoscandia. At least 20 years of data in the first half of the nineteenth century exist for the three stations, Övertorneå (66.4° N 23.8° E), Hailuoto Carlö (65.0° N 24.7° E) and Vöyri (63.2° N 22.0° E). The correlations between these early data and our reconstructed series are 0.64 (1802–1832), 0.48 (1817–1836) and 0.73 (1800–1824), respectively. These values would be expected to occur by chance one time in twenty in the case of Hailuoto Carlö and one time in a thousand for Övertorneå and Vöyri. There is also a very short early record (1830–1838) for Karesuando, which is near to the Torneträsk tree sites. The correlation between these temperature data and our reconstructions, 0.85, has a chance probability of below one in a hundred.

Summer temperature reconstructions

The complete reconstruction from 500 is plotted as temperature anomalies from the mean of 1951–70 in Fig. 2*a*. This long record shows that alternating periods of generally cool and generally warm conditions are typical of northern Fennoscandian summers throughout the last millennium. It is also clear that the length and amplitude of these temperature 'cycles' has varied dramatically during this time (Fig. 2*b–d*). There are only two periods (on a timescale of decades and above) when temperatures remained near the long-term mean: one lasting through the sixth and seventh centuries and a much shorter period in the latter half of the thirteenth century.

The smoothed line superimposed on the recent section of the reconstructed curve in Fig. 2*a* shows 10-year filtered values of the instrumental record for north Fennoscandia. This smooth line can be used as a modern yardstick against which to compare the magnitude and duration of summer temperature changes over the past 15 centuries. The relative warmth of the 1930s and 1940s is clear. Measured summer temperatures over these 20 years were on average 0.56 °C higher than the 1951–70 mean whereas the mean anomaly for the preceding 50 years (1880–1929) was −0.31 °C. The reconstruction shows that this recent cycle is part of a quasi 80–90 year oscillation which has continued since ~1700 (see Fig. 2*d*).

The mean of the reconstructed values for 1930–49 is 0.31 °C, and that for 1880–1929 is −0.20 °C. There are eight periods (based on 20-year running means) with warmth at least

TABLE 2 Calibration of reconstruction equation

Calibration Period		1876–1925	1926–1975	1876–1975
Verification Period		1926–1975	1876–1925	—
Calibration				
Explained Variance				
Overall		0.49	0.44	0.51
<10 years		0.49	0.53	0.51
>10 years		0.49	0.19	0.54
Verification				
Explained variance				
Overall		0.42	0.46	—
<10 years		0.48	0.49	—
>10 years		0.23	0.49	—
Reduction of error		0.56	0.59	—
Coefficient of efficiency		0.33	0.43	—
First-difference Sign test				
Correct signs		38	33	72
Incorrect signs		11	16	27
Regression weights (equation (1))	b_1	−0.026	0.122	0.081
	b_2	0.073	0.080	0.109
	b_3	0.675	0.536	0.600
	b_4	−0.014	0.113	0.052

Summary of the regression results for separate 50-year calibrations and the final reconstruction equation fitted over the full 100 years of overlap between the temperature and tree-ring data.

equivalent to 1930-49 persisting over two or more decades in the extended record, 750-780, 920-940, 960-1000, 1160-1190, 1400-1440, 1540-1570, 1750-1780 and 1840-1860. The warmest 20-year means occurred in the twelfth and the eighteenth centuries (Table 3). The second half of the twelfth century was also the warmest 50-year period (an anomaly of 0.35 °C over 1152-1201). Periods of cool conditions similar to those that prevailed in the later decades of the nineteenth century include 780-830, 850-870, 1110-1160, 1330-1360, 1570-1620 and 1800-1820. Interestingly, both the warmest 20-year period and the coolest 20-year period occur in the twelfth century. Both the coolest and warmest 50-year periods occur in this century (Table 3).

The cycle of temperature change that occurred in the twelfth century (cooling to ~1140, rapid warming to ~1160 and subsequent cooling) is the most notable feature of the reconstruction (Fig. 2). The reconstructed anomaly of 1.59 °C in 1092 was the warmest up to that date. The lowest value in the whole record, −1.65 °C, occurs in 1139. Between these dates temperatures decrease rapidly and almost monotonically. The largest 50-year cooling trend in the record, −1.78 °C, occurred between 1090 and 1139. After this an even more dramatic warming took place. Within 30 years temperatures rose by >2 °C, with anomalies >1.6 °C occurring in 1161 and 1164. The period with the largest positive 50-year trend in the reconstruction is 1127-1176 with a value of 2.43 °C. From ~1160 temperatures again decreased linearly, a 50-year trend of −1.76 °C being registered from 1161-1210 (the third largest 50-year cooling of the record). Although the largest oscillation in the record, the temperature cycle that occurred through the twelfth century is not unique. A similar cycle occurred in the second half of the eighth and the early part of the ninth centuries (Fig. 2*b*-*d*).

Medieval Warm Epoch and Little Ice Age

It is widely believed that two periods with strongly contrasting annual temperatures occurred during the late Holocene. The first, thought to have been a period of prolonged warmth, has been described as the Medieval Warm Epoch or the Little Climatic Optimum. It is believed to have lasted, in Europe at

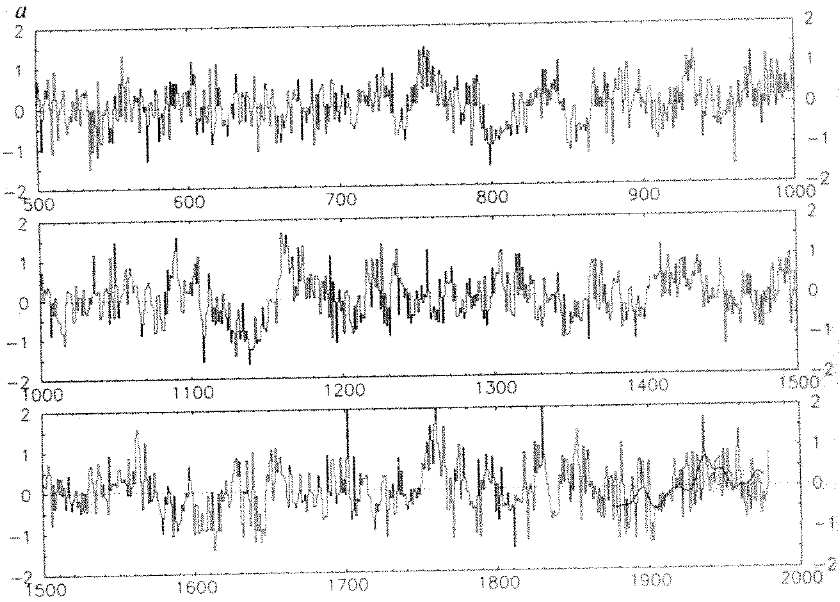
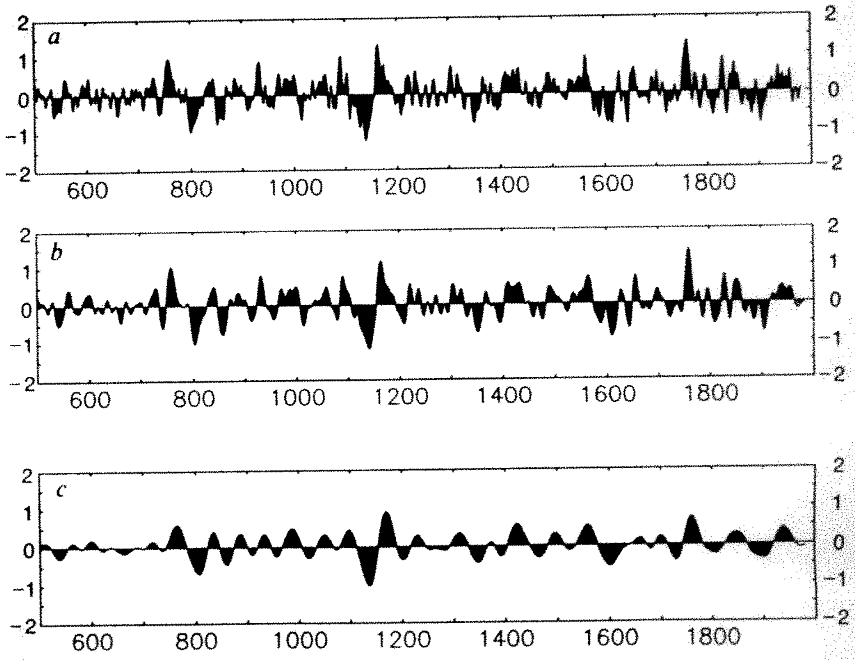


FIG. 2 *a*, Reconstructed northern Fennoscandian temperature anomalies (°C) (April-August mean) relative to 1951-70. Measured temperatures, smoothed with a 10-year low-pass filter, are also shown after 1876. *b*-*d*, The reconstructed summer temperatures after filtering with different gaussian and band-pass filters. *b*, 10-year low-pass values; *c*, variations between 25 and 40 years; *d*, variations between 50 and 150 years.



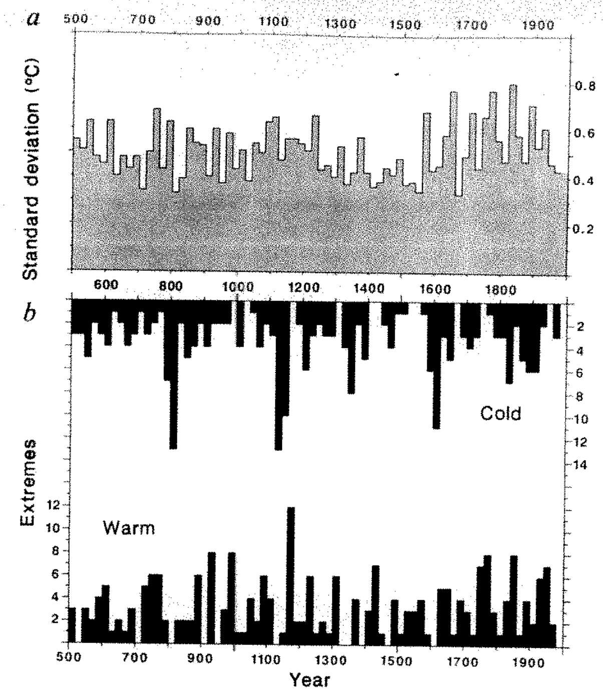


FIG. 3 *a*, Non-overlapping 20-year totals of negative and positive extremes. Extremes are defined as absolute anomalies ≥0.57 °C (one standard deviation of the full reconstructed series). *b*, Standard deviations calculated over the same periods.

least, from ~1000 to 1300[17]. Our reconstruction dispels any notion that summers in Fennoscandia were consistently warm throughout that period. Although the second half of the twelfth century was very warm the first half was very cold. For most of the eleventh and thirteenth centuries, summers were near normal (relative to the mean for 1951-70).

The other extensively cited period has been termed the Little Ice Age. During this time temperatures in many areas of both hemispheres are thought to have fallen to their lowest levels since the end of the last glaciation[17]. Perhaps not surprisingly, given the natural spatial variability of climate and the poor resolution of much of the data, there is confusion associated with the use of this term and there is considerable uncertainty as to the severity or synchroneity of the various cool events which have been ascribed to it[18]. Even in Europe the Little Ice Age has been said to have started at dates ranging between the twelfth to the sixteenth centuries and to have ended in the late seventeenth, the middle nineteenth and the early twentieth centuries[19]. There is, however, probably some concensus for a main European phase between 1550 and 1700 or 1800[17]. Our reconstruction offers strong evidence that summer temperatures in northern Fennoscandia did fall abruptly, to a level ~0.5 °C below the long-term mean, at ~1570. But they remained at this level only until 1650. Between 1600 and 1650, there were 13 summers with mean temperature anomalies below −1 °C. This is the largest number of such cold summers in any 50-year period in the reconstruction, approached only by the 12 that occurred between 1100 and 1150. The 1550s and the 1650s, however, were warm. Conditions were near normal from 1660 to 1750 and the 20 years between 1750 and 1770 were extremely warm, the equal warmest 20 years of the whole reconstruction (Table 3).

Much of the evidence for a Medieval Warm Epoch and the Little Ice Age comes from documentary historical data[17]. A more direct comparison with such data can be made by using counts of extremely warm or cold summers. When this is done (Fig. 3*a*), the results lead to essentially the same conclusions. Our reconstructed summer temperatures show little evidence for a Medieval Warm Epoch in Fennoscandia and the only evidence for the Little Ice Age comes from the period ~1570-1650. As regards the latter period, similar periods of cold summers occurred during the late eighth/early ninth century, the first half of the twelfth century and the second half of the fourteenth century. The first half of the twelfth century was almost certainly much more severe than the 1570-1650 period. Absence of a prolonged Medieval Warm Epoch or Little Ice Age in northern Fennoscandia is the most direct interpretation of our results. This implies either that northern Fennoscandia has experienced climate fluctuations that differed markedly from

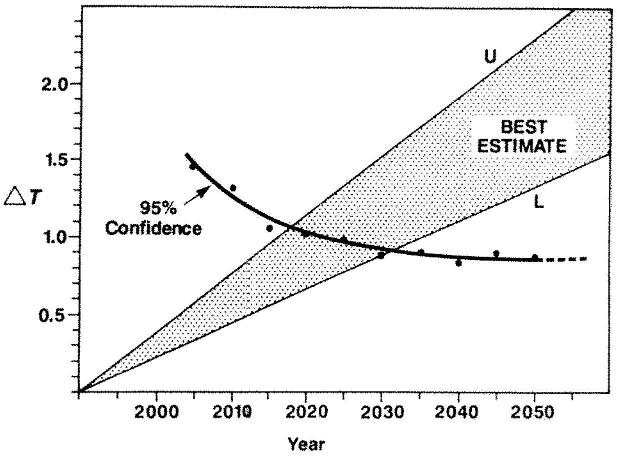


FIG. 4 The stippled area shows the 'best estimate' range of northern Fennoscandian summer temperature increase expected to result from increases in greenhouse gases (from ref. 21; some more recent estimates, unpublished, give lower values). The curved line can be considered a minimum threshold over which future summer temperature trends must pass before a greenhouse signal can be said to be distinguishable from natural climate variability at the regional scale. This detection threshold is drawn by hand through a series of empirically derived points each of which is the upper-95-percentile point of the distribution of trends of length up to that point on the horizontal axis. For example, the point plotted at 2030 (40 years after 1990) comes from the distribution of 40-year trends in the reconstruction. The illustrated threshold is a minimum because not all of the observed climate variance is reconstructed. Therefore, this graph probably provides lower-bound estimates of the detection time.

TABLE 3 Largest temperature anomalies and trends

Positive Temperature anomaly (°C)	Year	Negative Temperature anomaly (°C)	Year
Individual summers			
2.07	1761	−1.65	1139
2.05	1831	−1.58	1109
1.99	1703	−1.57	961
1.71	1937	−1.53	800
1.67	1161	−1.50	536
20-year means			
Positive Temperature anomaly (°C)	Period	Negative Temperature anomaly (°C)	Period
0.78	1158–1177	−0.93	1127–1146
0.78	1748–1767	−0.73	795–814
0.67	749–768	−0.65	1601–1620
0.52	1087–1106	−0.55	848–867
0.49	1551–1570	−0.53	1344–1363
50-year means			
Positive Temperature anomaly (°C)	Period	Negative Temperature anomaly (°C)	Period
0.35	1152–1201	−0.58	1108–1157
0.34	1403–1452	−0.42	1576–1625
0.33	1750–1799	−0.34	780–829
0.29	719–768	−0.31	1867–1916
0.27	962–1011	−0.23	1345–1394
50-year linear trends			
Positive Temperature change (°C)	Period	Negative Temperature change (°C)	Period
2.43	1127–1176	−1.78	1090–1139
1.77	1718–1767	−1.77	757–806
1.63	797–846	−1.76	1161–1210
1.17	850–899	−1.38	1560–1609
1.13	1385–1434	−1.27	1754–1803

the rest of Europe, or that the significance of these climate periods in Europe has been overstated, or both. In support of the latter possibility, we note that the proxy evidence for spatially coherent century-timescale climate fluctuations around the North Atlantic basin is relatively weak[18] and the historical evidence is equally sketchy owing to problems of data reliability[20].

Changes in interannual variability

It is commonly believed that the Little Ice Age was marked by high interannual temperature variablity. The same is often thought true of cool periods in general. We have tested this idea by calculating the standard deviations of non-overlapping 20-year periods (Fig. 3*b*) and comparing them with the means for the same 20-year periods. The expected relationship would yield a negative correlation. Actually, the correlation is positive (0.23) and marginally significant at the 5% level. The three reconsructed 20-year periods with largest standard deviations (in the range 0.77–0.80 °C) all have means that are either positive or near zero. The coolest reconstructed period during the Little Ice Age (−0.65 °C in 1601–20) has a standard deviation of only 0.45 °C. Interestingly, there does seem to have been a sharp increase in the magnitude and variability of the 20-year standard deviations for the period after the second half of the sixteenth century (Fig. 3*b*). This is, however, strongly accentuated by the distinctly low standard deviations for the 20-year periods in the preceding 300 years.

Greenhouse-warming detectability

Our extended reconstruction allows us to evaluate possible future changes in the climate of our study region in the context of natural variability, and so to address the issue of detection of greenhouse-gas-induced climate change at the regional level. We do this by comparing expected trends in summer temperature with those that have occurred over the past 1,481 years. A recent study[21] of possible greenhouse-gas-forced temperature change predicts that between 1990 and 2030 the global mean temperature will increase by 0.4–2.3 °C, with a 'best estimate' in the range 1.0–1.7 °C. Another study, integrating the regional patterns of climate change produced in a number of doubled CO_2 experiments using equilibrium general circulation models, suggests that the summer temperature change in northern Fennoscandia is likely to be somewhat less than this, between 0.75 and 1.0 times the annnual mean global change[22]. Therefore, a rough estimate of the expected summer temperature trend in northern Fennoscandia resulting from the greenhouse effect is somewhere between ~0.9 and 1.5 °C over the next 40 years.

We have used our reconstruction to generate the observed frequency distributions of trends over periods of different length up to 60 years, using only those trends that originate from the currently prevailing level, that is, within a range of temperatures 0.1 °C on either side of the long-term mean. This avoids distortions that may arise from warming and cooling trends that represent 'recovery' from very anomalous conditions. Plotting the upper-95-percentile point from each of the observed distributions of 15-, 20-, 25-, and so on, year trends, along with the range of predicted local greenhouse warming allows us to estimate when the predicted changes will rise above the 'noise' of natural variablity (Fig. 4).

Even if the climate sensitivity is at the top of the generally accepted range (U in Fig. 4), it will still not be possible to detect a regional greenhouse signal (with 95% confidence) until around 2020. If climate sensitivity is at the lower end of the range (L), as is indicated by the empirical evidence of recorded global temperatures over recent decades[23], then we will be unable to identify the signal in Fennoscandian temperatures with high confidence until some time after the year 2030. □

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Transient folding intermediates characterized by protein engineering

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Kinetic experiments on engineered mutants of barnase detect an intermediate on the folding pathway and allow the mapping of the tertiary interactions of the side chains and their energetics. Many of the interactions present in the final folded state tend to be either fully formed or not formed at all in the intermediate or subsequent transition state for folding, but the hydrophobic core becomes progressively consolidated. These methods in combination with NMR provide extensive structural characterization of the folding intermediate and the sequence of events in the folding pathway.

THE pathway of protein folding is dominated by noncovalent interactions that determine structure and stability. There is growing evidence that the refolding of proteins *in vitro* proceeds through intermediates that can accumulate transiently[1-5]. Unlike intermediates in covalent chemistry, which can frequently be isolated and characterized because of the relatively stable chemical bonds, the intermediates in protein folding, aside from -S-S- linkages, differ just in noncovalent bonding. The ephemeral nature of such bonds causes severe problems in the characterization of folding intermediates and requires novel methods for their investigation[6-9]. A recent high-resolution approach uses NMR, which can detect backbone NH groups that undergo hydrogen exchange slowly with solvent because they are hydrogen bonded within α-helices, β-sheets or other structural elements. Rapid quenching experiments on hydrogen exchange during refolding can detect secondary structure that is formed faster than the final folded structure. For example, regions of β-sheet and α-helix respectively, are formed rapidly in the refolding of RNase A (ref. 6) and cytochrome *c* (ref. 7). Earlier work also shows intermediate states. Spectroscopy and light and X-ray scattering measurements on α-lactalbumin have shown the existence of a state, termed the molten globule, which is stable under mildly denaturing conditions (acid pH, moderate concentrations of denaturants or high temperatures[1-5]). This state has significant secondary structure and is compact, but has a fluctuating hydrophobic core. It is difficult to study by NMR because of broad linewidths and extensive overlapping of peaks but methods have been developed to confirm some of its characteristics[8]. An intermediate state similar to or identical with the molten globule, has been suggested as a general occurrence on folding pathways[10,11].

We have applied protein engineering to analyse the unfolding of barnase, a small RNase from *Bacillus amyloliquefaciens*, and have shown how this approach can characterize the noncovalent structural changes in the transition state, which is inaccessible to the usual spectroscopic methods[12]. Mutations were made that remove individual interactions stabilizing different regions of the folded structure. Each mutation acts as a specific probe for structural changes during unfolding. We show here how kinetics can detect a transiently formed intermediate in the refolding pathway and present the full theory of how the protein engineering procedure can characterize structural properties of the intermediate that cannot be detected by NMR-hydrogen-exchange, such as events in the hydrophobic core or in hydrogen bonds that are in fast exchange, in addition to giving indirect evidence on secondary structure. The procedure also gives the energetics of noncovalent interactions within these conformations. The kinetic studies are complemented and reinforced by a separate study in which NMR-H-exchange experiments identify further secondary structure in the intermediate[13].

Kinetics detects folding intermediate

From experiment, the logarithm of the equilibrium constant for the unfolding of small proteins in urea (K_u) is proportional to the urea concentration[14]:

$$\log K_u = \log K_u^{H_2O} + m_e[\text{urea}] \qquad (1)$$

where $k_u^{H_2O}$ is the equilibrium constant for unfolding in water

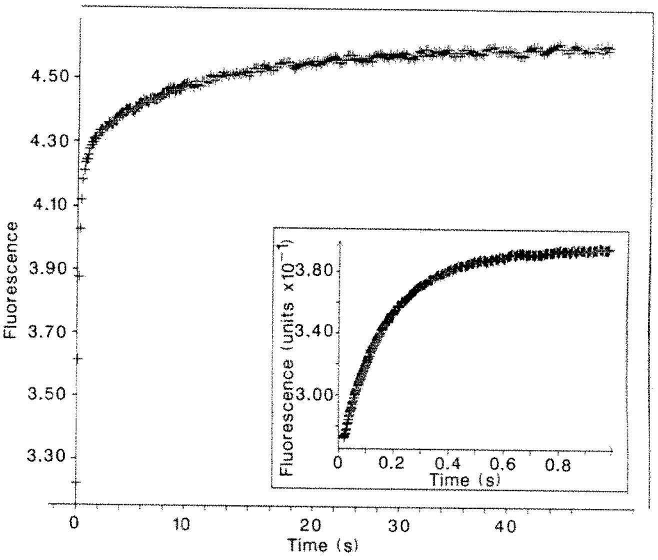

FIG. 1 Refolding of wild-type barnase monitored by increase in tryptophan fluorescence, excitation at 290 nm and emission at 315 nm. Refolding was initiated by mixing protein denatured in 7 M urea, 50 mM MES buffer, pH 6.3 with 10 volumes of 50 mM MES, pH 6.3 at 25 °C to give 0.636 M urea, using a Perkin-Elmer spectrofluorimeter MPF 44B fitted with a stopped-flow mixer[12]. The time scale for the slower phase is 0 to 50 s. Inset, fast phase from 30–950 ms. The fast phase has a rate constant of 6.5 s^{-1} and amplitude 0.131 units whereas the slow phase increases at 0.094 s^{-1} with amplitude 0.032 units. The rate constant for the slow phase varies only within a range of ±20% for different mutants under the same conditions. The thickness of the trace does not represent the noise in the signal but is an artefact of the symbols used by the plotter. The signal-to-noise ratio is better than 50:1. The amplitude of the slow phase is that expected if ~5% of each peptidyl prolyl linkage is *cis*. A value of 0.044 to 0.053 has been recently reported for the equilibrium constant for *cis/trans* Lys 116–Pro 117 in unfolded staphylococcal nuclease[24,25].

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and m_e is a constant that is proportional to the increase in degree of exposure to solvent of the protein on denaturation. The rate constant for unfolding, k_u, is generally found to increase with increasing urea concentration according to equation (2) where $k_u^{H_2O}$ is the rate constant in H_2O (refs. 5,12–15).

$$\log k_u = \log k_u^{H_2O} + m_{ku}[\text{urea}] \qquad (2)$$

Equations (1) and (2) imply that the rate constant for folding, k_F, of a protein that involves the reversible transition between just two states, E_F and E_U, (equation (3))

$$E_F \underset{k_F}{\overset{k_u}{\rightleftharpoons}} E_U \qquad (3)$$

must follow the rate law

$$\log k_F = \log k_F^{H_2O} - (m_e - m_{ku})[\text{urea}] \qquad (4)$$

(As $\log k_F^{H_2O} = \log (k_u^{H_2O}/K_u^{H_2O})$, the value of $\log k_F$ may be calculated simply from the unfolding rate constants and equilibrium data.)

The kinetics of protein folding is often complicated by the slow and frequently rate-determining isomerization of proline residues[16]. Barnase has three proline residues, which are all *trans* in the native structure. Earlier work suggests that some 5–10% of each of the prolines in the denatured protein should exist in the *cis* conformation and that the conformations should interconvert with a half-life of tens of seconds and an activation energy of about 15 kcal mol^{-1} (ref. 17). We find that when barnase that has been incubated in 6–8 M urea is diluted with 10 volumes of water there is a biphasic regain of folded structure with the two kinetic phases well resolved (Fig. 1). The first phase, 83% of the amplitude measured by stopped-flow fluorescence spectroscopy[12], occurs with a half-life about 50 ms at low urea concentration that increases with increasing urea. The slow phase, 17% of the amplitude, has a half-life of 10–20 s, changes only slowly with urea concentration and has an activation energy of about 13 kcal mol^{-1}. These are just the characteristics expected for the conversion of ~5–10% each of three proline residues in the *cis* conformation in the unfolded enzyme, to *trans* in the folded. (This interpretation has subsequently been confirmed directly by showing that the slow phase is efficiently catalysed by peptidyl prolyl isomerase, L. Meiering, unpublished results). The fast and main phase is the folding of the fraction of unfolded protein that has all its prolines in the *trans* conformation in solution. Measurements of regain of activity on renaturation demonstrate that the fast phase corresponds to the formation of active enzyme. The following discussion refers just to the fast phase.

Figure 2 shows that whereas the simple rate law of equation (1) describes the unfolding of wild-type barnase, $\log k_F$ does not follow equation (4). The value of $\log k_F$ at low urea concentration deviates by many orders of magnitude from that predicted by the kinetics of a two-state equation. This is classic evidence for the existence of a metastable intermediate on the refolding pathway: the intermediate accumulates on a fast time scale and the observed rate constant is that for the reaction of the intermediate and not for its formation. The deviation from ideality is not caused by the equilibrium and rate equations (1) and (2) breaking down at lower concentrations of urea. The evidence for this is: $\log k_u$ can be measured with suitable mutants under more acidic conditions down to 0.5 M urea and is linear with urea concentration over the relevant concentration range (Fig. 2); $K_u^{H_2O}$ measured from the linear extrapolation of

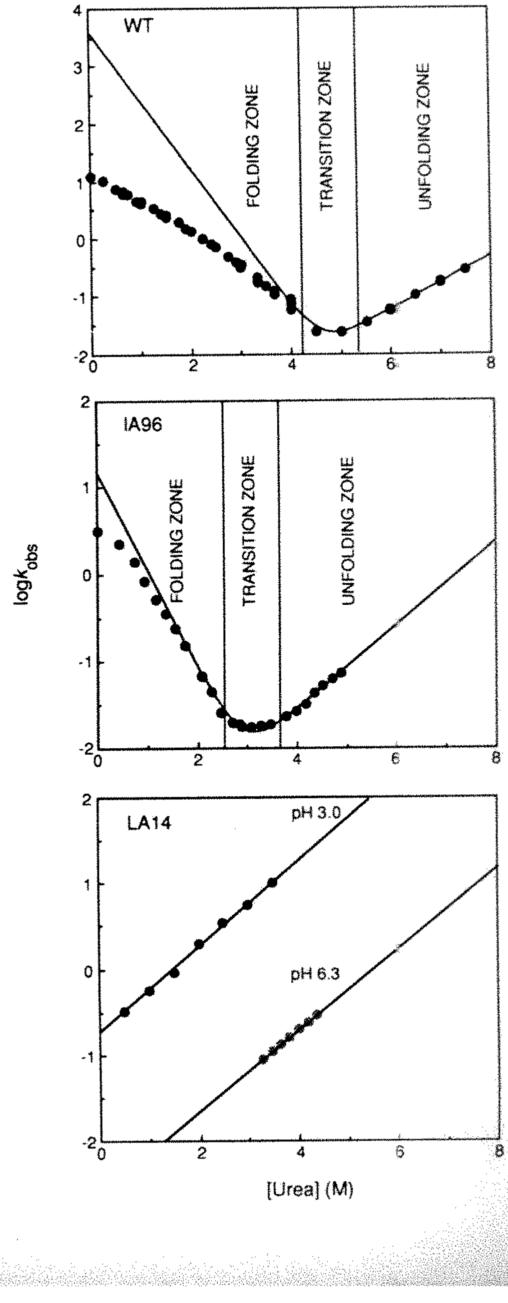

FIG. 2 Urea dependence of rate constants for denaturation and renaturation of wild-type and mutant enzymes. Rate constants are measured in units of s^{-1} at 25 °C, pH 6.3 and 50 mM MES buffer. Upper panel, $\log k_{obs}$ for folding of wild-type enzyme. The experiments at low concentrations of urea consist of solutions of enzyme in high concentrations of urea diluted 11-fold into refolding buffer in the stopped-flow fluorimeter[12]. In this region, k_{obs} is the rate constant for refolding. (The point at 0 M urea was obtained from the renaturation of acid-denatured enzyme, after a pH jump from low pH.) At high urea concentration, folded enzyme in aqueous buffer was mixed with 10 volumes of urea solution, to observe the kinetics of unfolding. In the transition region, both folded and unfolded protein exist at measurable concentrations. The rate constants in this region may be measured by mixing either unfolded or folded protein with urea and monitoring the progress to equilibrium ($k_{obs} = k_u + k_F$ for a simple two-state transition when both forward and reverse rate constants are significant)[26]. The solid curve is that calculated for a simple two-state system (equation (3)) and $\log k_{obs} = \log (k_u + k_F)$ (ref. 26). The rate constant k_u was calculated from the experimental values for the unfolding kinetics, which conform to equation (2) with $k_u^{H_2O} = 1.1 \times 10^{-4}$ s^{-1} and $m_{ku} = 0.463$ M^{-1}. k_F was calculated by inserting the value of k_u into equation (1) using the values of $K_u^{H_2O}$ and m_e from equilibrium unfolding measurements (The two-state approximation holds in the transition region). Middle panel, $\log k_{obs}$ for the mutant IA96 (Ile → Ala). The two-state approximation holds nicely down to less than 2 M urea and well below the transition region. The solid curve was calculated as above using $k_u^{H_2O} = 3.2 \times 10^{-4}$ s^{-1} and $m_{ku} = 0.479$ M^{-1}. Bottom panel, $\log k_u$ measured at low concentrations of urea for the destabilized mutant LA14 (Leu → Ala). The stability of barnase decreases at low pH (ref. 27). At pH 3.0 LA14 is >95% unfolded at 0.5 M urea. LA14 was dialysed against distilled water at pH 7 and mixed in the stopped-flow fluorimeter, as above, with 10 volumes of 110 mM sodium formate, pH 3.0, containing various concentrations of urea. The data for pH 6.3 are given for comparison.

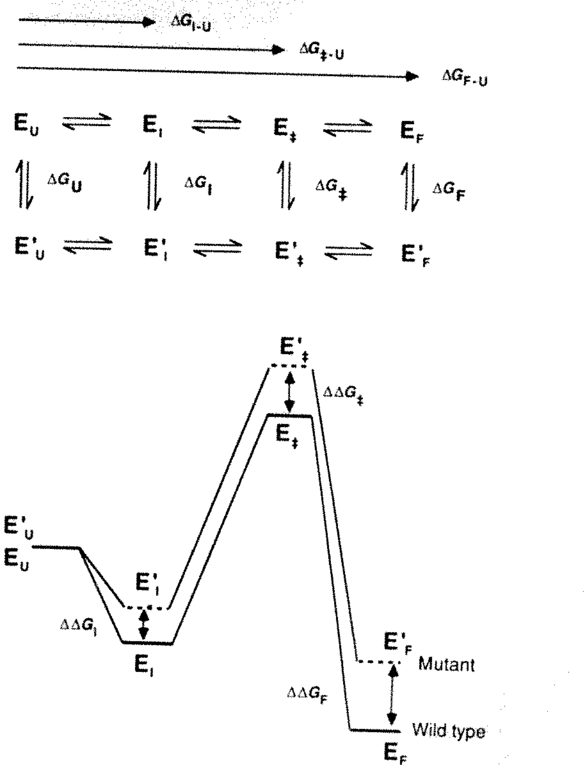

FIG. 3 Thermodynamic analysis. Top, Cycles relating properties of mutant and wild-type enzymes. The energy difference between E_F and E_U, $\Delta G_{F\text{-}U}$ $(= G_{E_F} - G_{E_U})$, is measured from equilibrium unfolding. The energy difference between E_F and E_I, $\Delta G_{F\text{-}I}$ $(= G_{E_F} - G_{E_I})$, is calculated from the ratio k_u/k_{-u}. $\Delta G_{I\text{-}U}$ $(= G_{E_I} - G_{E_U})$ is calculated from $\Delta G_{F\text{-}U} - \Delta G_{F\text{-}I}$. The free-energy of the transition state, $\Delta G_{\ddagger\text{-}F}$ $(= G_{E_\ddagger} - G_{E_F})$ can be calculated from transition state theory[26] and the value of k_u, the rate constant of unfolding[12]. $\Delta G_{\ddagger\text{-}U}$ $(= G_{E_\ddagger} - G_{E_U})$ is calculated by adding $\Delta G_{\ddagger\text{-}F}$ and $\Delta G_{F\text{-}U}$. There may be uncertainties in calculating the absolute values of $\Delta G_{\ddagger\text{-}F}$ by transition state theory but the errors cancel out when measuring $\Delta\Delta G_\ddagger$, the difference in $\Delta G_{\ddagger\text{-}F}$ between wild-type and mutant enzymes, as only ratios of rate constants are used. For wild-type enzyme, the following values are calculated in H_2O at 25 °C and pH 6.3: $\Delta G_{I\text{-}U} = 3.2$ kcal mol^{-1}; $\Delta G_{\ddagger\text{-}U} = 9.6$ kcal mol^{-1}; and $\Delta G_{F\text{-}U} = -10.2$ kcal mol^{-1}. In the vertical virtual equilibria, ΔG_U is the difference in noncovalent energy between unfolded wild-type and mutant enzymes, $\Delta G_I = G_{E_I} - G_{E'_I}$ is the difference between the folding intermediates, $\Delta G_\ddagger$ is the difference between the transition states and ΔG_F between the folded states. Bottom, Calculation of difference energies. The difference energies are defined as: $\Delta\Delta G_F = \Delta G_{F\text{-}U} - \Delta G'_{F\text{-}U}$; $\Delta\Delta G_\ddagger = \Delta G_{\ddagger\text{-}U} - \Delta G'_{\ddagger\text{-}U}$; $\Delta\Delta G_I = \Delta G_{I\text{-}U} - \Delta G'_{I\text{-}U}$ where the prime denotes mutant. In practice, all energies are calculated as difference energies and relative to the folded state ($\Delta\Delta G_{F\text{-}U}$, $\Delta\Delta G_{F\text{-}I}$, $\Delta\Delta G_{F\text{-}\ddagger}$) and then converted to the difference energies relative to the unfolded state by subtracting them from $\Delta\Delta G_{U\text{-}F}$. The difference energies are the experimentally determined values. These may be related to the true differences in noncovalent energy between wild-type and mutant proteins by extending theory developed previously[12,21] (see text). The difference energies may be measured as above with high precision. $\Delta\Delta G_F$ measured from equilibrium denaturation is reproducible to ±0.03 kcal mol^{-1}. The value of $k_{-u}^{H_2O}$ is measured to better than ±5% (standard error). The precision of values of $k_u^{H_2O}$ vary from a standard error of ±14% for wild-type from the extrapolation to 0 M urea of experiments conducted at concentrations above 5.5 M, to ±2% for the shorter extrapolation of unfolding data for a destabilized mutant such as LA14 (see Fig. 2). The consequent errors in $\Delta\Delta G_\ddagger$ and $\Delta\Delta G_I$ are estimated from the method of calculation to be less than ±0.14 kcal mol^{-1}. The differences between $\Delta\Delta G_\ddagger$, $\Delta\Delta G_I$ and $\Delta\Delta G_F$ are even more precisely known because $\Delta\Delta G_\ddagger$ and $\Delta\Delta G_I$ are measured relative to $\Delta\Delta G_F$. The error in $\Delta\Delta G_\ddagger - \Delta\Delta G_F$ and in $\Delta\Delta G_\ddagger - \Delta\Delta G_F$ and is less than ±0.11 kcal mol^{-1}.

measurements of log K_u at high urea concentration agrees with that measured by temperature- or guanidinium chloride-induced denaturation[18]; the folding kinetics of some less stable mutants follow the two-state equation down to much lower concentrations of urea because the intermediate is destabilized and accumulates to a much smaller extent (for example, mutant IA96 in Fig. 2).

It has been suggested that the deviation in the rate constants for refolding is not a consequence of an intermediate accumulating but is caused by the *cis* → *trans* isomerization of one of the proline residues becoming rate determining at low urea. This is unlikely for the following reasons. First, the amplitude of the fast phase (83%) is so high that it would require the *cis* conformation to be the major form in the unfolded enzyme. Second, the rate constant of 13 s^{-1} is far too high for a *cis-trans* isomerization of a proline in a denatured protein. Third, the high amplitude accounts for most of the reaction and so there would still be a discrepancy between the equilibrium constant for unfolding calculated from the ratio of rate constants and that from calculated equilibrium studies.

The NMR study[13] shows directly that on the folding pathway there is an intermediate with considerable formation of secondary structure. The refolding of wild-type enzyme is a multiphasic reaction of which we measure only the slowest steps in the stopped-flow experiments. The faster steps are lost in the dead time of the stopped-flow spectrophotometer, which is 30 ms because of problems in mixing urea with water. The slowest step is the isomerization of prolines in the denatured state. This step has the same rate constant for all the mutants studied. The following analysis of the kinetic consequences of the folding intermediate on the refolding pathway applies to the phase that is observed in the stopped-flow time range before the *cis-trans* isomerization.

Free energy profile for folding

The role of binding energy of many groups at the active site of the tyrosyl-transfer RNA synthetase was elucidated by comparing the free-energy profiles of wild-type and mutant enzymes throughout the reaction[19]. What is crucial is not so much the free-energy profile itself but the differences in energy levels between mutant and wild-type enzymes. The 'difference energy' diagrams readily give a qualitative picture of the importance of each side chain throughout the reaction[19,20] and the results can be interpreted quantitatively[21]. The same approach may be applied to study the energetics of interactions of side chains during protein folding.

First, the free-energy profile of the folding pathway of the wild-type protein must be (partly) constructed. The kinetics of folding from 30 ms fit, with high precision, a single exponential trace with a slow tail for proline isomerization[13]. There may be many intermediates on the reaction pathway. Some of these occur before the formation of the one observed, E_I, but are lost in the dead time of the apparatus because they are formed and react too fast. Others will occur between E_I and E_F but are after the rate-determining step for folding (see discussion in ref. 12). The observed rate constant, k_{-u}, is simply that for E_I transforming to E_F (equation (5)). The observed rate constant for unfolding, k_u (equation (2)), is the rate constant for the formation of E_I from E_F, which is the rate-determining step for unfolding.

$$E_U \rightleftarrows \cdots \rightleftarrows E_I \underset{k_u}{\overset{k_{-u}}{\rightleftarrows}} E_F \qquad (5)$$

(If 'E_I' is a mixture of intermediates, then k_{-u} is a weighted mixture of rate constants, and reports back on the weighted average of the properties of the mixture.)

The schemes for analysing the kinetics and equilibria are given in Fig. 3. The analysis is in terms of difference energy diagrams or apparent energy differences. The free-energy profile for folding of wild-type enzyme is calculated from the measured free energy of unfolding and the rate constants $k_u^{H_2O}$ and $k_{-u}^{H_2O}$, the values in the absence of denaturant, to give the energies of the folded state, transition state for unfolding and the intermediate relative to the unfolded state. The unfolded state is assigned a standard energy of 0. The same is done for a mutant. An accurate procedure for calculating the differences in the energy levels is given in the legend to Fig. 3.

Difference energy diagrams

In Fig. 4 are plotted the difference energy diagrams for a variety of mutants, each chosen as before[12], to remove a small interaction that stabilizes a specific part of the structure. The crucial step in the interpretation is to relate the experimentally measured difference energies ($\Delta\Delta G_I$, $\Delta\Delta G_\ddagger$ and $\Delta\Delta G_F$ in Fig. 3) to the true differences in noncovalent energy between each of the states. The true differences are defined as:

$$\Delta G_U = G_{E_U} - G_{E'_U}, \qquad \Delta G_I = G_{E_I} - G_{E'_I},$$
$$\Delta G_\ddagger = G_{E_\ddagger} - G_{E'_\ddagger} \quad \text{and} \quad \Delta G_F = G_{E_F} - G_{E'_F}$$

(where G_{E_U} is the free energy of unfolded wild-type enzyme, $G_{E'_U}$, that of unfolded mutant, and so on). Applying the first law of thermodynamics to the cycle in Fig. 3 gives:

$$\Delta\Delta G_F = \Delta G_F - \Delta G_U \tag{6}$$

$$\Delta\Delta G_\ddagger = \Delta G_\ddagger - \Delta G_U \tag{7}$$

$$\Delta\Delta G_I = \Delta G_I - \Delta G_U \tag{8}$$

The apparent energies ('$\Delta\Delta G$') differ from the true by a constant term for each mutant, ΔG_U, the difference in free energy between unfolded mutant and wild-type enzymes. An important corollary of equations (6)-(8) is that the change in the experimentally determined apparent energies on going from one state to another in the folding profile is equal to the difference in true energies. For example, from equations (6) and (7):

$$\Delta\Delta G_F - \Delta\Delta G_\ddagger = \Delta G_F - \Delta G_\ddagger \tag{9}$$

and from equations (7) and (8):

$$\Delta\Delta G_\ddagger - \Delta\Delta G_I = \Delta G_\ddagger - \Delta G_I \tag{10}$$

Changes in apparent energies ($\delta\Delta\Delta G_{\text{app}}$) are always equal to changes in true stabilization energies ($\delta\Delta G_{\text{true}}$) along a profile, that is, $\delta\Delta\Delta G_{\text{app}} = \delta\Delta G_{\text{true}}$. Equations (6)-(10) hold for all mutations in all circumstances. But they apply to the global energies of the different states of wild-type and mutant enzymes. The crucial question in the interpretation of these equations is, therefore, whether the changes in energy relate just to the local changes in bond energies around the site of mutation. It is possible that $\delta\Delta\Delta G_{\text{app}}$ has a significant contribution from longer range global changes that vary from state to state between wild type and mutant. This can be analysed by dividing the change in noncovalent energy on mutation of a side chain into two terms: one, ΔG_{local}, arising from changes where the mutated side chain was previously in contact with other residues; and a second, ΔG_{reorg}, the reorganization energy[21], arising from changes in the energy of the protein elsewhere, in both conformational energy and solvation. Thus, $\delta\Delta\Delta G_{\text{app}} = \delta\Delta G_{\text{local}} + \delta\Delta G_{\text{reorg}}$. If $\delta\Delta G_{\text{reorg}} = 0$, then, $\delta\Delta\Delta G_{\text{app}}$ reports back on just the local events during folding. If, $\delta\Delta G_{\text{reorg}} \neq 0$, then $\delta\Delta\Delta G_{\text{app}}$ is uninterpretable in a simple manner.

The two major assumptions in this study are: the mutations are such that ΔG_{reorg} and $\delta\Delta G_{\text{reorg}}$ are small and do not obscure the results; and the mutations do not perturb the pathway of folding. This achieved by using two categories of mutation. The first is deletion where a portion of a side chain is removed without introducing a new function (such as Ile → Val, Tyr → Phe). If there are no changes in protein structure on mutation other than removal of part of the side chain, the mutation is said to be nondisruptive[20] and $\delta\Delta G_{\text{reorg}}$ is zero. Under these conditions, $\delta\Delta\Delta G_{\text{app}} = \delta\Delta G_{\text{local}}$. Further, where there is an empty cavity in the mutant enzyme at the site of deletion, $\delta\Delta G_{\text{local}}$ is a direct measure of the interaction energy of the side chain in the wild-type enzyme with the contiguous residues. Mutations that cause an increase in the size of a buried side chain or add new contacts are avoided as they are likely to lead to disruptions of structure and the high probability that $\delta\Delta G_{\text{reorg}} \neq 0$.

The second category is mutation of surface residues. Here, solvent can move to accommodate changes. Further, for changes at the surface where there is free access of water to the mutated sites, $\Delta\Delta G_{\text{app}}$ approximates to the true binding energy of the group that is deleted[21]. We prefer to make mutations that give relatively small changes in stabilization energy as not only are they likely to cause insignificant values of ΔG_{reorg} and $\delta\Delta G_{\text{reorg}}$, they are also the least likely to perturb the pathway of folding.

We find for many of the mutations analysed in this study that there is a greatly simplifying feature: the change in stability of the intermediate or transition state on mutation is either close to zero or close to 100% of that of the change in stability of the folded enzyme. These are the changes expected if the interactions are, respectively, either not formed at all or fully formed in the intermediate or transition state.

The difference energies are calculated with high precision. For each mutant relative to wild type, the error in $\Delta\Delta G_F$ is less than ± 0.03 kcal mol^{-1}, and in $\Delta\Delta G_\ddagger$ and $\Delta\Delta G_I$ less than ± 0.14 kcal mol^{-1} (see legend to Fig. 3). Relative to $\Delta\Delta G_F$, $\Delta\Delta G_\ddagger$ and $\Delta\Delta G_I$ are known to an accuracy of better than ± 0.11 kcal mol^{-1} as the error in $\Delta\Delta G_F$ affects the three energy terms equally. Problems in interpreting difference energies thus do not arise from uncertainties in the accuracy of the measurements.

Analysis of mutants

YF78, TA6, TA26–probing a loop and the N termini of helices. Tyr 78 stabilizes a major loop by its -OH group forming hydrogen bonds with the NH and C=O of Gly 81 in the folded state (Fig. 5). Mutation of Tyr → Phe (YF78) is a deletion that should not cause significant structural changes. It was shown previously[12] that in this mutant all the stabilization energy from the hydrogen bonds is lost in the transition state for unfolding. All the energy is lost in the intermediate as well: $\delta\Delta\Delta G_{\text{app}}$ is almost zero between E_I and $E_\ddagger$. The -OH of Thr 26 acts as the N-cap of the second helix, forming hydrogen bonds to the NH groups of residues 27–29 (ref. 22). Mutation of the N-cap to Ala (TA26) is a good mutation to analyse as it is simply an alteration of a surface residue that is replaced by solvent water. Again, it was shown previously by mutating TA26 that all the energy of the N-cap is lost in the transition state for unfolding and the same is seen to happen in the intermediate. Thr 6 forms the N-cap of the first helix. In the mutant TA6 (Thr → Ala) 80% of the difference energy is lost in the transition state for unfolding, and the same amount of energy is lost in the intermediate as in the transition state.

HQ18, TS16–probes of the major α-helix. The charge on the protonated form of His 18 makes a coulombic interaction with the dipole of the α-helix from residues 6–18, and an imidazole NH makes a hydrogen bond with the backbone CO of Gln 15. The interaction energy is thus a probe of the integrity of the C terminus of the helix. In the mutant HQ18(His → Gln), most of this interaction energy is maintained in the transition state[12]. It is maintained equally in the intermediate. A second residue that probes the C terminus is Thr 16. The γ-methyl of Thr 16 makes a very strong hydrophobic interaction with the aromatic ring of Tyr 17, the next residue in the helix in the folded structure. In the mutant TS16 (Thr → Ser) this is maintained in the transition state for unfolding[12], and is seen now to be equally maintained in the intermediate, adding further evidence for early helix formation. These mutations are most suitable as they alter just surface side chains that are exposed. Under these circumstances, values of $\Delta\Delta G_{\text{app}}$ are close to the true binding energies[21].

LA14, IA88, IA96, IV88, IV96–probes of the hydrophobic core. The principal α-helix (residues 6–18) packs onto the antiparallel β-sheet by the interdigitation of hydrophobic side chains, of which Leu 14, Ile 88 and Ile 96 are representative, to form a hydrophobic core. Mutation of these differs from the previous

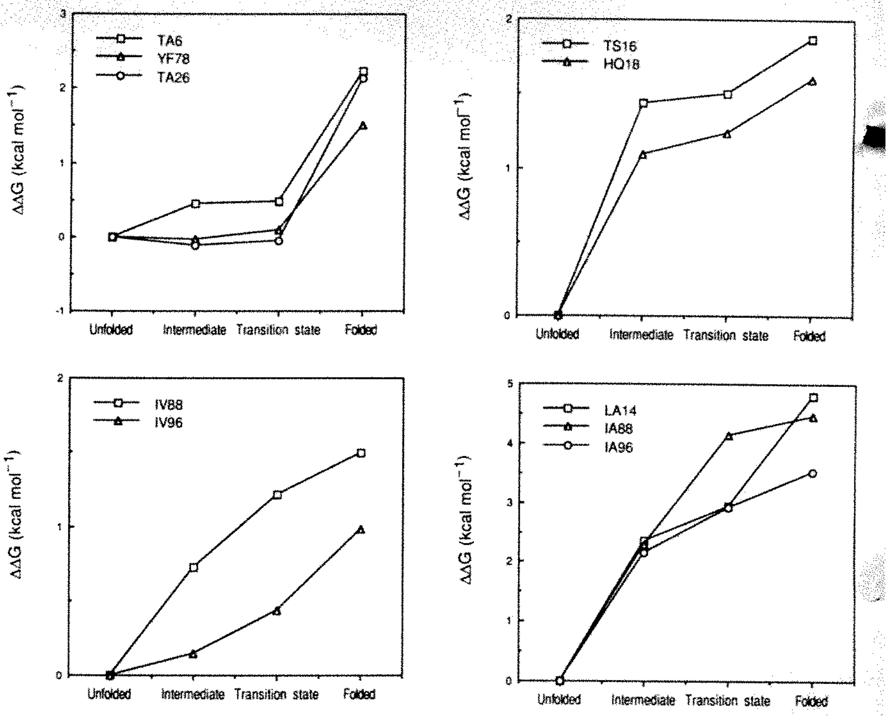

FIG. 4 Difference energy diagrams. The difference energies, $\Delta\Delta G_{app}$, calculated according to Fig. 3 are plotted for the mutants. The following rate constants and equilibrium data have been used.

Wild type: $k_u^{H_2O}=1.1\times10^{-4}\ s^{-1}$, $k_{-u}^{H_2O}=13\ s^{-1}$;
TA6: $k_u^{H_2O}=2.2\times10^{-3}\ s^{-1}$, $k_{-u}^{H_2O}=15\ s^{-1}$;
TA26: $k_u^{H_2O}=4.6\times10^{-3}\ s^{-1}$, $k_{-u}^{H_2O}=14\ s^{-1}$;
YF78: $k_u^{H_2O}=1.2\times10^{-3}\ s^{-1}$, $k_{-u}^{H_2O}=13\ s^{-1}$;
TS16: $k_u^{H_2O}=2.1\times10^{-4}\ s^{-1}$, $k_{-u}^{H_2O}=14\ s^{-1}$;
HQ18: $k_u^{H_2O}=2.1\times10^{-4}\ s^{-1}$, $k_{-u}^{H_2O}=12\ s^{-1}$;
LA14: $k_u^{H_2O}=2.7\times10^{-3}\ s^{-1}$, $k_{-u}^{H_2O}=5.6\ s^{-1}$;
IV88: $k_u^{H_2O}=1.8\times10^{-4}\ s^{-1}$, $k_{-u}^{H_2O}=6.5\ s^{-1}$;
IA88: $k_u^{H_2O}=2.0\times10^{-4}\ s^{-1}$, $k_{-u}^{H_2O}=0.7\ s^{-1}$;
IV96: $k_u^{H_2O}=2.9\times10^{-4}\ s^{-1}$, $k_{-u}^{H_2O}=9.2\ s^{-1}$;
IA96: $k_u^{H_2O}=3.2\times10^{-4}\ s^{-1}$, $k_{-u}^{H_2O}=4.1\ s^{-1}$.

Identical values for $k_{-u}^{H_2O}$ are obtained from either extrapolation of the rate constants for refolding at low urea concentrations to 0 M or directly from renaturation by a pH jump from pH 1.5 to 6.3 in the absence of urea. $k_u^{H_2O}$ is obtained by linear extrapolation (see data in ref. 12). A referee has noted that the values of $\Delta\Delta G_F$ for just the interactions measured here account for most of the observed free-energy of folding of the protein ($\Delta G_{F\text{-}U}=-10.2$ kcal mol^{-1}). This is not anomalous because, as defined in Fig. 3, $\Delta G_{F\text{-}U}=G_F-G_U$. G_F and G_U are both much larger than $\Delta G_{F\text{-}U}$, possibly being in the region of thousands of kcal mol^{-1}. Thus, the values of $\Delta\Delta G_F$ are <1% of G_F. Whether or not a protein folds depends on the small difference between G_F and G_U. Just a few mutations can tip the balance so that the unfolded form is favoured at equilibrium.

examples as there are obvious progressive changes in the difference energies as the protein folds (Fig. 4). Mutation of these apolar side chains should lead to small values of ΔG_U (ref. 12) and so changes in difference energies most probably do reflect the true energy changes. Mutations of isoleucines to valines (IV88, IV96) are better probes than mutation to alanine (IA88, IA96) as the smaller the mutational change the less the perturbation of structure. Ile→Val is almost an ideal mutation whereas Ile→Ala leaves a larger cavity. Surrounding residues could relax into the cavity with some probability of a significant reorganization energy term, or there could be ingress of solvent. For IV96 and IV88, there is a clear progression of $\Delta\Delta G_{app}$ as the reaction proceeds, and the same is true for the larger mutations. There are individual variations in the energetics but, in all cases, the stabilization of the intermediate is about midway between that of the unfolded and folded states whereas the transition state has up to 80–90% of the final stabilization. The individual variation might reflect the differences in binding of the different methylene groups in each side chain or could be a consequence of ΔG_{reorg} terms. Nevertheless, it is clear that the hydrophobic core becomes consolidated as folding proceeds.

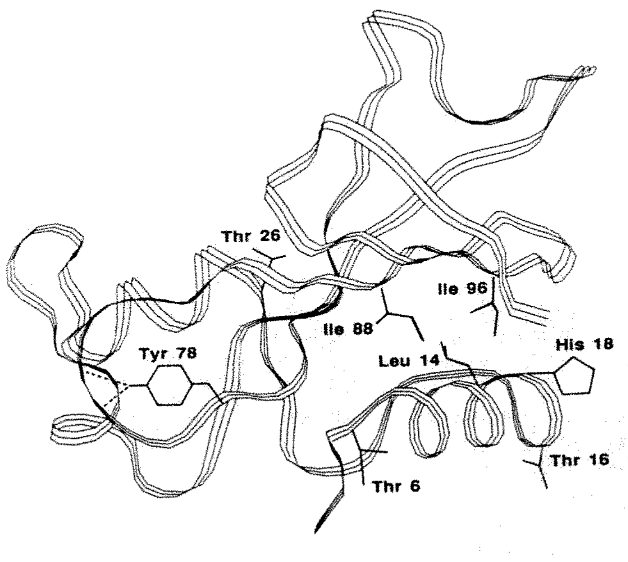

FIG. 5 Sketch of barnase illustrating the target residues.

Reliability of structural conclusions

The deductions about changes in structure from changes in energetics are, of course, indirect. Aside from the assumptions that $\delta\Delta G_{reorg}$ is small and the mutations do not perturb the pathway of folding, what other problems could there be to obscure the interpretations? The results can be divided into two extreme classes to illustrate these: those where interactions are maintained on changing state and those where interactions are lost. Maintenance of an interaction energy does not prove that the particular interaction itself is maintained; it could be that a different interaction of equivalent energy is formed in the new state. Total loss of an interaction energy in an intermediate, however, is conclusive evidence for the loss of an energetically significant interaction as there is not the ambiguity of an alternative interaction. The evidence for loss of the N-caps during the unfolding of barnase is, thus, unambiguous. Evidence for maintenance of the interactions at the C terminal of the major helix is very good. Two separate mutations, based on different properties of the helix, give consistent results, as do NMR experiments on hydrogen exchange[13]. Results on the hydrophobic core are less conclusive; the changes in interaction are intermediate between the two classes. The core is unambiguously weakened in the transition state and intermediate, but it could be changed even more than indicated by the mutational data as there could be alternative hydrophobic interactions taking place.

Intermediate and folding events

Protein engineering on Thr 16 and His 18 shows that the C-terminal portion of the α-helix extending from residues 6–18 is formed in the intermediate. N-capping of the two larger helices occurs after the rate-determining transition state for folding as

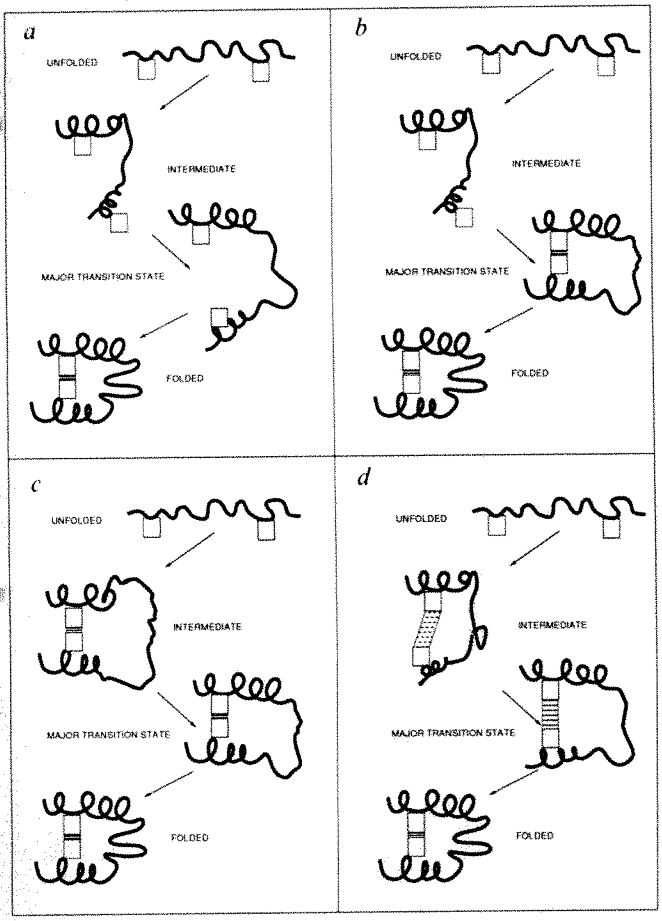

FIG. 6 Possible steps in the folding of barnase. *a*, Two residues do not interact in the unfolded, intermediate or transition states but form after the rate-determining step. Mutation of these does not affect the kinetics of folding but alters the unfolding rate constant. Examples of this are the N-caps and Tyr 78. *b*, The residues interact in the transition state and folded state. No examples yet found. *c*, Interactions occur from the intermediate onwards. Examples approximating to this are His 18 and Thr 16 interacting with residues in the major α-helix. *d*, Progressive bond formation, as with the hydrophobic core.

does the closing of the loop containing Tyr 78. The hydrophobic core between the 6-18 helix and the antiparallel β-sheet consolidates as folding proceeds, implying that the antiparallel β-sheet must be present to some extent. Much of the structural detail of the intermediate is the same as that deduced earlier for the transition state[12], the most striking difference between the intermediate and transition state discovered so far being the weaker hydrophobic core. The weak hydrophobic interactions in the intermediate may be due either to the core being fluid and weakly packed or to the intermediate being composed of an equilibrium mixture of many states, some of which are well packed and others not. In either case, formation of the final folded structure from the intermediate requires the precise docking of elements of the preformed secondary structure to give a closely packed core and intact loops. Interestingly, the interactions studied here, apart from those in the core, are either close to being fully formed or not formed at all in the transition state or intermediate (Fig. 6). NMR-H-exchange[13] confirms the existence of the secondary structure and shows that the C termini of all three α-helices are formed in the intermediate as are many of the interactions in the sheet.

Earlier work on stable molten globule intermediates showed general evidence for secondary structure and qualitative evidence for a weakened hydrophobic core[1-9]. This description fits the intermediate found on the folding pathway of barnase. But NMR studies on the molten globule state of α-lactalbumin, which has extensive secondary structure and a condensed hydrophobic core, show only a few NH groups that are significantly protected against exchange[2,9] whereas the NH groups in barnase are extensively protected. The protein engineering methods suggest changes that can be made to stabilize the intermediate in barnase so that it can be studied directly by NMR. Certain mutations, such as those in the loops and N-caps, destabilize the folded structure but not the intermediate. Judicious choice of a combination of these mutations should destabilize the native folded state to such an extent that the intermediate should become the predominant compact state at low concentrations of urea and so may be studied directly. Kinetic studies on further mutants should enable us to map most of the structure of the intermediate as well as that of the transition state[12].

Our results show the strengths and limitations of the NMR and protein engineering methods, which are in many ways complementary. NMR gives detailed information about the extent and timing of formation of secondary structure. Protein engineering gives detailed information about the extent, timing and strengths of formation of tertiary interactions, and indirect evidence about secondary structure. Protein engineering is more general as most side chains can be readily modified, and it can be applied to proteins which are too large to be examined by NMR. Kinetics is the only method that can be used to study transition states. Both methods are indirect, as interactions are deduced rather than observed directly. But the consistency of results between the two methods implies that they are giving correct information.

One approach to predicting tertiary protein structure is to predict the repeating secondary structures, such as α-helices and β-sheets, and then dock them (see ref. 23). Our results suggest that docking of preformed elements is indeed part of the rate-determining process in folding and provides encouragement for that theoretical approach. □

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Understanding recent observations of the large-scale structure of the universe

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RECENT galaxy redshift surveys[1-3] offer evidence of a quasi-periodic network of 'sheets', with typical dimensions of $\sim 100h^{-1}$ Mpc (h is the Hubble constant in units of 100 km s^{-1} Mpc^{-1}). Galaxy formation models in which large-scale structure forms purely by gravitational instability from small initial irregularities seem to have problems producing structures on such large scales without violating constraints on the anisotropy of the microwave background, unless they start from apparently contrived initial-fluctuation spectra. Models in which galaxies and clusters have a non-gravitational (possibly explosive) origin seem to be better candidates for the progenitors of large sheets and bubbles, but one would still not expect a perfectly regular lattice. So how surprising are the observations, if they are interpreted in the framework of such models? Here I show that a random cellular model, which mimics the galaxy distribution formed as matter is swept up into shells around expanding void regions or by cosmic blast waves, reproduces the observed Great Walls, voids and periodic structures without undue difficulty.

The most exciting recent developments in observational cosmology have been the results obtained from the surveys of galaxy redshifts[1-3]. Unfortunately, only partial information about the true three-dimensional distribution of galaxies is obtained from such surveys because of difficulties in obtaining a complete sample. Wide-angle surveys can only reach out to relatively low redshifts so attempts to study the distribution out to much higher redshifts must use a thin almost one-dimensional, sample volume (for example, see ref. 3). Results from the Center for Astrophysics (CfA) survey[1,2] give the impression of a rather 'bubbly' structure or possibly a pattern of intersecting 'sheets' in the planes of which most galaxies lie. Because of the relatively short range of this wide-angle survey, one cannot see many of these structures and their significance is difficult to assess, although they have typical sizes of at least several tens of megaparsecs. The remarkable results of Broadhurst *et al.*[3] seem to confirm the sheet structure because they find a very highly clustered distribution of galaxies along a single line of sight through space, qualitatively consistent with what one would expect to see if one drilled through a bubbly pattern. What is remarkable, however, is the regular spacing of the clumps along the line of sight (to ~ 10% of the mean spacing). Given that virtually all models of galaxy formation involve some sort of random initial conditions, it is very difficult to see how any such regular pattern could have evolved. It is therefore important to see if any reasonable stochastic clustering models can reproduce the observations.

One of the simplest methods of generating stochastic cellular patterns is to use a geometric tessellation model. The most useful planar or volumetric tessellation is the Voronoi tessellation[4,5], sometimes called the Dirichlet or Thiessen tesellation. In three dimensions the Voronoi tessellation consists of space-filling network of Voronoi cells, each of which is a convex polyhedron. A cell is defined by its nucleus and contains the region of space that is nearer to its own nucleus than to any other. The simplest version of the Voronoi model, which I use here, is based on a random spatial distribution of nuclei, that is, the nuclei form a spatial Poisson point pattern, characterized by a mean number density n. Figure 1 shows a two-dimensional Voronoi tessellation. Properties of the tessellation in three dimensions are considerably more complicated than those of the two-dimensional analogue (for example, the mean number of vertices of a two-dimensional Voronoi cell is just 6, whereas a three-dimensional Voronoi cell has 27.07). Various authors[6-10] have studied this and related models, concluding that: (1) if galaxies populate the faces of the Voronoi cells, then the model reproduces the qualitative pattern of cells and sheets found in the observations;[1] (2) if rich clusters of galaxies form at vertices of the tessellation, where more than two cells meet—this is where very high overdensities would be expected to form—then the observed correlation function of rich Abell clusters can be reproduced[8] if $n \simeq (100h^{-1}\ \mathrm{Mpc})^{-3}$; (3) there is strong physical motivation for the Voronoi tessellation as an approximation to the matter distribution formed at late times in many physical models, whether the driving force is provided by explosions centred on the Voronoi nuclei or by gravitational expansion of underdensities (in the latter case, the Voronoi nuclei represent peaks of the initial peculiar gravitational potential and the tessellation thus formed traces out the large-scale pattern produced by N-body evolution to a remarkable accuracy[6]. None of these conclusions is drastically altered if the Voronoi nuclei are moderately clustered[7,8], showing that the applicability of the construction is not dependent on a particular choice of initial conditions, so the results I obtain for the simple Poisson version of the model are quite general. Of course, some galaxies will form outside the cell surfaces, or may move off them once they have formed, so the model can tell us very little about the distribution of galaxies within or in the immediate vicinity of a 'sheet'. One can regard the Voronoi tessellation as a good model for the spatial distribution of the sheets themselves, however, and therefore as an important complement to traditional N-body techniques, which inevitably suffer from problems of sampling and boundary effects on such large scales.

Despite its apparent simplicity and its extensive use (for example, in stereology[11]) many of the geometric properties of the Voronoi tessellation were not known analytically until recently. Møller[12] has derived some important new results concerning the Voronoi tessellation and has investigated the properties of two-dimensional slices and one-dimensional lines of sight through a three-dimensional Voronoi foam. Without giving the mathematical derivation, I demonstrate below the importance of these results for the interpretation of clustering data.

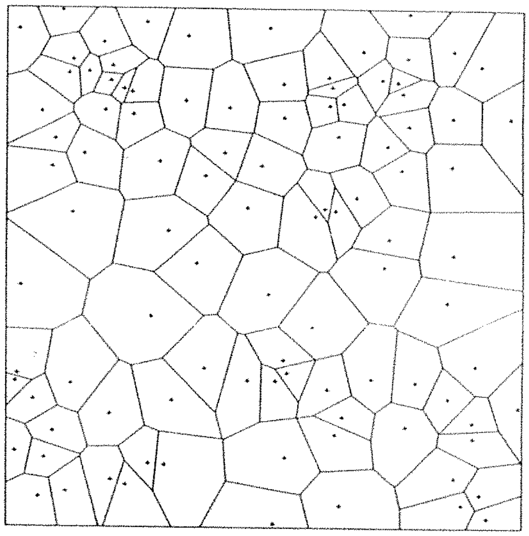

FIG. 1 An example of a two-dimensional Voronoi tessellation generated by a random spatial distribution of 100 nuclei. Although the tessellation is generated by a totally random point set, it exhibits a surprising degree of regularity, particularly in the sizes of the larger cells. This regularity is even more pronounced in three-dimensional tessellations and would be enhanced even further by the destruction of small cells by nonlinear dynamics.

First I consider a line of sight through a Voronoi foam. Such a line will intersect with faces of different cells at (random) spacings λ_1. Møller has shown that, regardless of the distribution of nuclei

$$\langle \lambda_1 \rangle = 4\langle V \rangle / \langle A \rangle \tag{1}$$

where V and A are the volume and surface area of a randomly selected cell, and the angle brackets denote expectation values). In the simple Poisson model, this can be calculated exactly

$$\langle \lambda_1 \rangle = \frac{(81/4n\pi)^{1/3}}{\Gamma(2/3)} \tag{2}$$

where $\Gamma(x)$ is the Gamma function. Using the favoured value of n, $(100h^{-1}\ \mathrm{Mpc})^{-3}$, leads to $\langle \lambda_1 \rangle \simeq 137h^{-1}$ Mpc, remarkably close to the spacing of clumps observed by Broadhurst *et al.*, $\lambda \simeq 128h^{-1}$ Mpc; the disparity between these values is well within the uncertainty introduced by using Abell cluster correlations to fix n (ref. 8). Of course, the mean clump spacing is not the whole story. The Broadhurst *et al.* data display a set of eight or nine spacings with a dispersion less than 10% of the mean. Is this surprising? Using a very weakly clustered null model, they suggested that the probability of such an occurrence is $\sim 3\times10^{-4}$. Such problems are very difficult to solve rigorously, for two reasons. First, various complicated selection effects are involved: our Galaxy is presumably on a cell wall in this model; the four other fields in the original study of Broadhurst *et al.*[13] do not show such a regular pattern, imposing an *a posteriori* selection on the field that exhibits the structure; the line of sight goes through the Coma supercluster which is known to be a region of extremely high density; neighbouring Voronoi cell volumes are not independent; the smaller cells would be erased by nonlinear dynamics. The second problem is that higher-order moments of the properties of Voronoi cells are not known and these are required to construct a significance test. Møller does, however, give results for the moments of volumes of cells of a related tessellation, called the Delaunay tessellation, which is known to have similar sampling properties to the Voronoi. Scaling the moments of this distribution to the mean Voronoi cell volume gives an estimate of the moments of the distribution of Voronoi cell volumes. The probability that a sample of nine cells leads to a set of line-of-sight intersections with standard deviation <10% of the mean can now be calculated. It is a well-known result (see, for example, ref. 14) that the variance of the variance of a sample of n observations drawn from a population with k-th order central moments μ_k is $(\mu_4-\mu_2^2)/n$. Using the estimates of the moments μ outlined above, I can use this to give a rough estimate of the probability of obtaining a sample variance as small as that observed from nine cells. The result, admittedly very approximate, is ~5%, much higher than the result quoted by Broadhurst *et al.*[3] which was due to the lack of any large-scale clustering in the null model they used.[3] It is clear that the selection effects outlined above will ensure that this result is a rough lower bound on the actual probability of reproducing the observations in ref. 3, so I conclude that if one uses a test model that reproduces the known cluster correlation function, the significance of the observed structures decreases substantially. Also only one out of five independent fields in the study[13] showed this marked regularity; this may be indicative of a probability of the order of that calculated above. The low significance level can be understood visually by looking at the apparent regularity of the tessellation in Fig. 1: this regularity in cell size increases with the dimensionality of the tessellation. Of course, the only way to simulate the observations reliably is with a Monte Carlo technique: such a study is in progress and seems to be giving results in broad agreement with the rough estimates given here (V. Icke, personal communication).

One can also use Møller's results to look at the properties of the CfA data[1,2]. The most striking feature of these results is the presence of a 'great wall' with dimensions $\sim 20\times60\times100h^{-1}$ Mpc. In the Voronoi model, this would be interpreted as a face of a Voronoi cell, with the smallest dimension being the thickness of the face caused by the motion of matter out of the plane defined by colliding shock fronts. The mean area of such a face F is

$$\langle F \rangle = \frac{35(2^8\pi)^{1/3}\Gamma(2/3)}{(9n)^{2/3}(24\pi^2+35)} \tag{3}$$

Using the favoured value of n, I calculate $\langle F \rangle \simeq 3{,}800(h^{-1}\ \mathrm{Mpc})^2$, rather smaller than the $6{,}000(h^{-1}\ \mathrm{Mpc})^2$ for the Great Wall. But this is not too difficult to explain—as the wall is appreciably curved, it may be two or more adjacent faces of the tessellation.

Next, I consider 'slices' such as the preliminary CfA survey[1], which comprised a roughly two-dimensional section through the spatial distribution. This data has a bubbly appearance and includes a void region with diameter $\sim 50h^{-1}$ Mpc. The mean area of a cell of the two-dimensional tessellation obtained by slicing a three-dimensional Voronoi tessellation is

$$\langle S \rangle = \frac{15}{\Gamma(1/3)(16\pi^5 n^2/9)^{1/3}} \tag{4}$$

giving $\simeq 6{,}860(h^{-1}\ \mathrm{Mpc})^2$, which is roughly comparable to the Bootes Void[15] and bigger than the voids seen in ref. 1.

I therefore conclude that the features observed in galaxy redshift surveys are not particularly improbable if the large-scale galaxy distribution follows a simple Voronoi tessellation. At first sight, one might think that the most obvious way of producing such a large-scale pattern physically is in an explosion model[10], but it provides a reasonable approximation to the distribution of matter on large scales even for gravitational instability models such as cold dark matter[16,17]: most theoretical calculations of large-scale clustering take account only of the linear growth of overdensities and, as argued by Icke[18], it may be the underdensities that are the dynamically important factor—after all, most of the Universe is actually in the voids[7]. The simulations in refs 16 and 17 seem to produce structures similar to those reported[1-3] and void expansion may well be the reason for this, although the microwave background places severe constraints[19]. Further theoretical work is needed. We need to know how many lines of sight produce a regular pattern like the one observed. The angular size of a typical Voronoi cell face at $z=0.2$ is ~1°, so a pencil beam aimed only a few degrees away from the one that produced the regular spacing should produce a much less regular pattern of intersections. Until this is shown not to be the case, the data can be said to fit fairly comfortably within a physically plausible model. ☐

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Spatial structures in microtubular solutions requiring a sustained energy source

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MICROTUBULES are believed to be the principal organizers of the cell interior[1]. Cells respond to a variety of stimuli by modifying the spatial distribution of the microtubules. These effects are central to cell division and morphogenesis[2], and embryo development[3]. During embryo development, macroscopic patterns are frequently observed[3]. Here we report that microtubular solutions spontaneously form alternating white and dark stripes about 1 mm wide and 1 cm long. Small-angle neutron scattering measurements show that in each segment the microtubules are aligned obliquely to the direction of the stripe, and that the white and dark stripes differ in having mutually orthogonal orientations. The formation of these structures requires an initial reservoir of organic phosphate. Phosphorus NMR measurements show that the process is accompanied by the energy-liberating conversion of organic to inorganic phosphate. These observations, together with similarities to the dissipative spatial structure formed by the Belousov–Zhabotinski reaction[4–6], provide strong evidence that the observed structures are energy-dissipative in nature. Dissipative structures are thought to be critical to the appearence of complex living organisms[7,8]. Our results strongly suggest that microtubules are capable of forming such structures. Microtubular dissipative structures may occur during mitosis and embryo morphogenesis.

Tubulin was purified from recycled bovine brain microtubule protein using standard phosphocellulose chromatography[9]. This preparation is known not to contain any significant amounts of impurities. We dissolved the tubulin in heavy water (D_2O) so as to obtain an adequate neutron scattering signal. Microtubules were formed by warming the tubulin (3–20 mg ml^{-1}) from 4 °C to 33 °C in the presence of 1 mM GTP, 20 mM acetyl phosphate and acetate kinase (1.25×10^{-3} mg ml^{-1}); their formation, monitored by turbidity and neutron scattering, was completed after ~15 min. At this stage, the samples appear uniform but over a period of 3–10 h, a series of horizontal white and dark stripes develop, which gradually encompass the whole cell (Fig. 1). The stripes are strongly birefringent. Once formed the structure remains stable for >24 h. On cooling to 4 °C the stripes disappear and the sample becomes transparent. On rewarming to 33 °C they reappear. These observations, together with the neutron scattering results discussed below, show that the stripes arise from microtubules. They are best viewed in reflected light, with the light coming to the cell obliquely (45°). Turning the cell by 90° interchanges the white and dark regions and rotating the cell by 45° minimizes the contrast. The samples were contained in quartz cells with optical path lengths of 1 or 2 mm; patterns of the same type were observed in cells of path length 0.5–5 mm.

We used small-angle neutron scattering[10] to investigate the microtubular structure of the stripes. Figure 2 shows contour plots of the neutron counts detected with a 64×64 two-dimensional array. The distribution of scattered intensity is highly asymmetric with most of the intensity concentrated into an arc, oriented at ~45° or 135° to the horizontal for the dark and white stripes, respectively. Plots of scattered intensity taken along these arcs show the scattering profile of microtubules[11,12] as viewed along their cross-section, and the spectra from the different stripes are of equal intensity. These results show that both stripes arise from equal concentrations of microtubules; the fact that the scattering is concentrated into an arc, and not a circle, indicates that the microtubules are aligned with their long axis perpendicular to the direction of scattering. The stripes are therefore regions of microtubules, alternately oriented at ~45° and 135° to the horizontal. This was confirmed by the scattering pattern using a vertical slit covering several stripes, which showed arcs at both 45° and 135°, consistent with the sum of the spectra from both orientations. The spots in Fig. 2 are not symmetrical, but show a trail towards the horizontal, consistent with a preferred misalignment of the tubes towards the vertical. This is the behaviour expected at the interface between stripes, where tubes of a 45° orientation form an interface with tubes of a 135° orientation. The microtubules in each segment showed no positional ordering between themselves, hence each stripe constitutes an orientated nematic liquid-crystalline phase.

This structure also explains the optical observations, because the tubules will strongly scatter light when viewed down their length, but will only scatter weakly, and therefore appear dark, when viewed across their diameter. Thus, the contrast is best when illuminated obliquely, and the light and dark regions are interchanged when the sample is rotated by 90°. Moreover, a stripe that is white when illuminated by light at 45° should show neutron scattering spots, arising from the microtubular cross-section, at 135° to the horizontal, as is observed. Also because the microtubular orientations are 45° and 135°, the stripes observed through crossed polarizing filters, at 0° and 90°, are of uniform birefringence, except for a line of extinction where the orientation changes. This corresponds to the observations shown in Fig. 1*b*.

Above a critical concentration, anisotropic objects such as long rods may spontaneously orient as a result of packing considerations[13]. Although this might explain the orientational ordering of the microtubules in each stripe, it does not account for the alternating mutually orthogonal orientations. The spontaneous formation of macroscopic periodic ordering is a property of nonlinear systems that are far from equilibrium[4–8,14–18]. Formation of these structures requires continual energy consumption or dissipation to drive the system and maintain it in a state of higher order and lower entropy than that arising under equilibrium conditions. The energy may be provided internally, by a chemical reaction such as in the Belousov-Zhabotinski reaction or externally, as in the application of a temperature gradient to a liquid layer (Bénard instability)[6,7,15], or the application of electric and magnetic fields to liquid-crystal films[19,20]. Under certain conditions, systems of the first type can generate chemical oscillations in time. Such oscillating chemical reactions have been observed for microtubules[21–23]. Microtubule formation and stability require the continual energy-liberating hydrolysis of GTP to GDP[23–25]. The overall process is thermodynamically irreversible[24] and is thought to be nonlinear[22,23]. Microtubules are thus associated with the properties required for the formation of dissipative structures.

The requirements for a structure to be termed dissipative are that the system be thermodynamically irreversible with a net input of energy, and that there be an energy source for the formation and maintenance of the structure. To provide a continuous source of GTP at constant concentration, it is common practice to add a solution of acetyl phosphate together with a GTP regenerating enzyme[26]. The formation and maintenance of the microtubules results in a net reaction in which acetyl phosphate is gradually converted into inorganic phosphate.

To provide direct evidence that the same process occurs in this system we took ^{31}P NMR spectra at different times during and after the formation of the striped structures. Spectra taken at different times are shown in Fig. 3. The three low-intensity peaks on the right arise from GTP and the two resonances on the left arise from inorganic phosphate and acetyl phosphate. With increasing time, the peak arising from inorganic phosphate increases at the expense of that from acetyl phosphate. Except

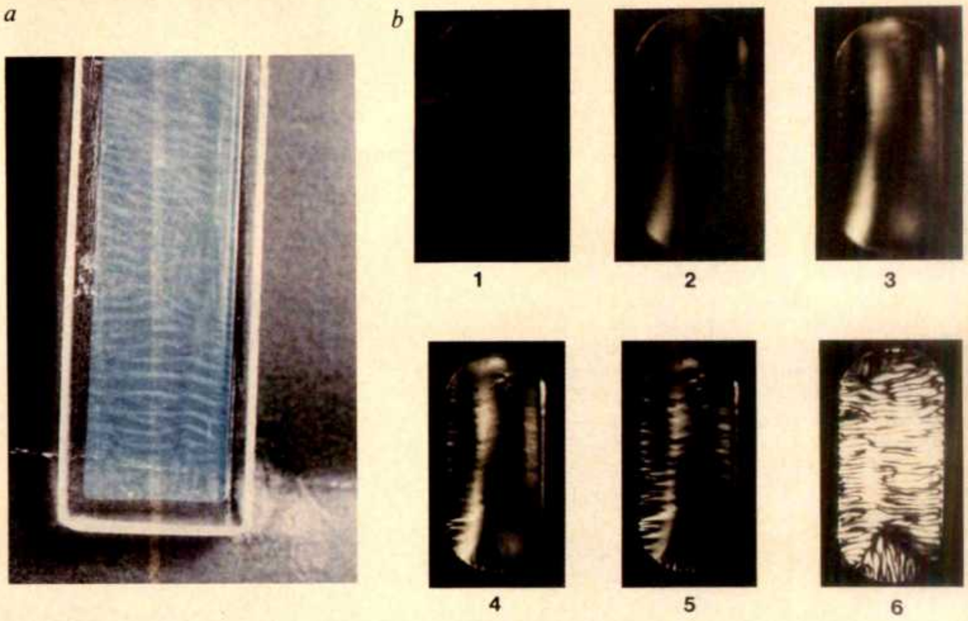

FIG. 1 *a*, Striped patterns formed by microtubular solutions at 33 °C observed in reflected light. The sample cell dimensions were 40 × 10 × 1 mm. The sample comprised tubulin (10 mg ml^{-1}) and GTP (1 mM) in a D_2O buffer containing 100 mM MES (2-*N*-morpholino ethanesulphonic acid), 1 mM EGTA (ethylene glycol-bis-(B-aminoethyl ether) *N,N,N',N'* tetra-acetic acid), and 1 mM $MgCl_2$ at pH 6.75. Microtubules continually dissipate energy by transforming GTP (guanosine triphosphate) to GDP (guanosine diphosphate). A regenerating system of acetyl phosphate (20 mM) and acetate kinase (1.25 × 10^{-3} mg ml^{-1}) was added to provide a continuous source of GTP. *b*, Microtubular solution, observed through crossed polarizing filters, at different times after initiating microtubule formation by warming from 4 °C to 33 °C; 1, 1 min; 2, 20 min; 3, 2h; 4, 3 h; 5, 4 h; 6, 13 h. The sample composition was the same as in *a* except that the tubulin concentration was 4.6 mg ml^{-1}, and the acetate kinase concentration was 5 × 10^{-4} mg ml^{-1}. The optical path length was 2 mm. The length of the microtubules during and after pattern formation was determined, using known procedures[21] to be ~5 μm. These patterns have been reproduced many times in different locations and environments, indicating that they do not arise from uncontrolled external factors such as vibrations. The appearance of the microtubular stripes shows a strong resemblance to the establishment of the dissipative space structure in the Belousov–Zhabotinski reaction.

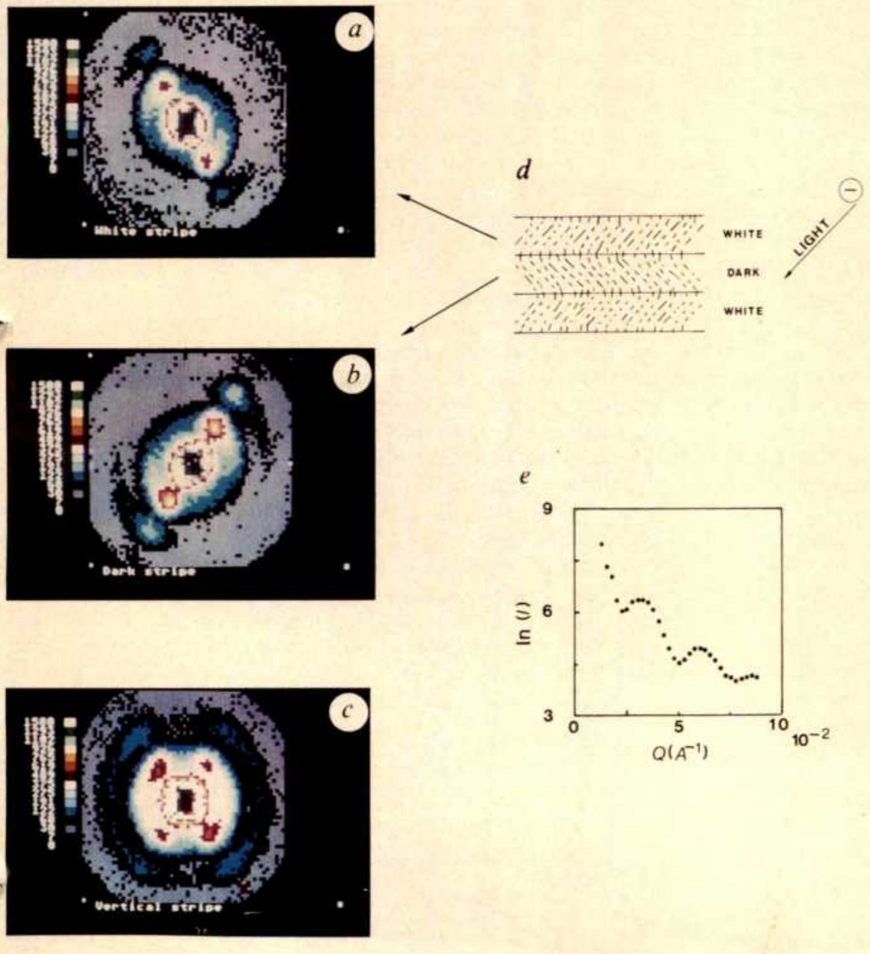

FIG. 2 Neutron scattering spectra from *a*, a white stripe, *b* a dark stripe, *c*, a vertical slit across several stripes (same sample as in Fig. 1*a*, top left corner). The different neutron-bombarded regions were preselected using a cadmium mask with a slit ($\frac{1}{2}$ × 6 mm) at different places on the front of the sample cell. Spectra were measured on the DII camera[32] (Institut Laue-Langevin) using a 2.5-m sample–detector distance and neutrons of 10-Å wavelength. Counting times were ~2 h. The results are shown as contour plots of the number of scattered neutrons on a 64 × 64 two-dimensional detector. The neutron beam, stopped by a rectangular cadmium plate after transmission through the sample, is centred on the detector. *d*, Schematic representation of the periodic microtubular structure formed. The microtubules are represented as dark rods. *e*, Scattering profile taken along one of the arcs, corresponding to the spectrum from microtubules[11,12] viewed along their cross-section. The momentum transfer Q is defined as $4\ \pi/\lambda\ \sin\theta$ where λ is the neutron wavelength and 2θ the scattering angle.

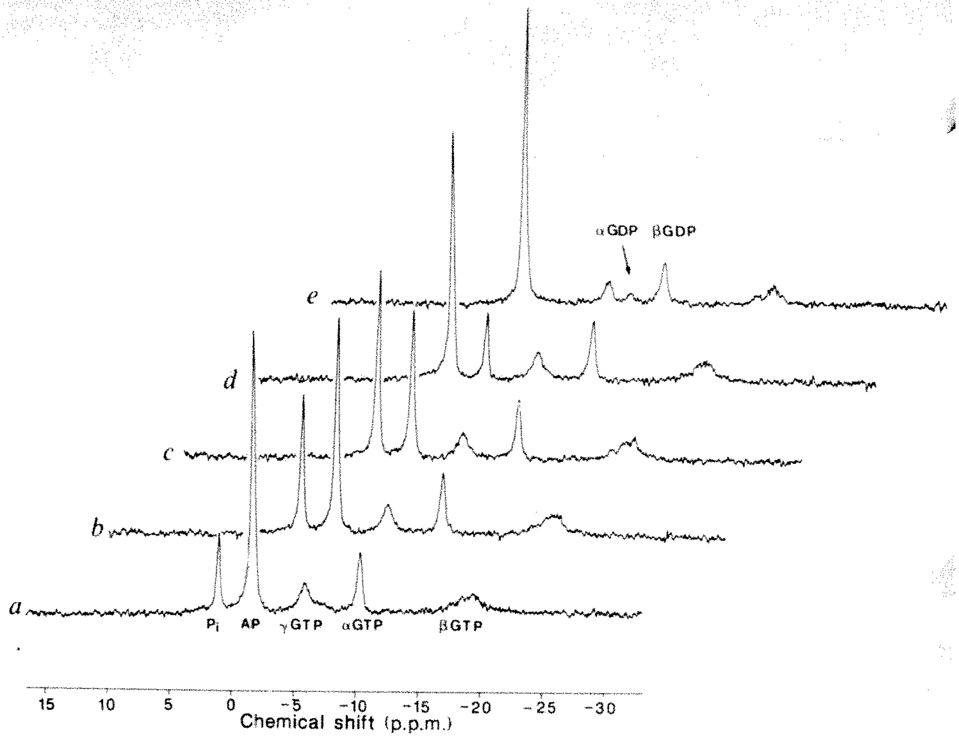

FIG. 3 ^{31}P (162 MHz) NMR spectra measured at different times during and after the formation of the dissipative structure: *a*, 40 min; *b*, 100 min; *c*, 200 min; *d*, 400 min; *e*, 24 h. The sample was contained in a 15-mm tube without spinning. Individual spectra took 13 min to accumulate. Chemical shifts are relative to an external sample of H_3PO_4. The resonance marked P_i and AP arise from inorganic phosphate and acetyl phosphate respectively. The position of the β-GDP resonance coincides with that of α-GTP.

for the last spectrum, the intensity of the GTP signals remains the same, signifying a constant concentration. When the acetyl phosphate is exhausted, the acetate kinase can no longer recycle the GDP, and after ~24 h a signal from GDP starts to appear. At this stage the kinetics are very slow, and the striped aspect and birefringence of the sample slowly begin to disappear; this is paralleled by a decrease in the total mass of polymerized tubulin until complete denaturation of the sample occurs.

The spectra shown in Fig. 3 confirm the reaction scheme outlined above and show that the formation of the striped structure is accompanied by an irreversible chemical reaction. As the hydrolysis of acetyl phosphate to inorganic phosphate is exothermic, energy is dissipated into the system. To show that this energy is essential to the formation and maintenance of the structures we prepared a series of samples containing 1 mM GTP and of acetyl phosphate concentrations varying from 0 to 20 mM. With 1 mM GTP and no acetyl phosphate, no stripes were formed. Stripes begin to form and become more marked as the acetyl phosphate concentration is increased. Turbidity measurements showed that, to within 10%, the same mass of microtubules were formed for all the samples. Also with increasing acetyl phosphate concentration, the structures were maintained for progressively longer periods. These results show that the acetyl phosphate is necessary for the formation and maintenance of the structures and at the same time constitutes an energy reservoir which is irreversibly consumed during the process.

The NMR results show that the rate of acetyl phosphate consumption is much higher during the formation of the stripes than after. Experiments of this kind should therefore permit the separation of the energy required for structure formation and structure maintenance. Raising the concentration of magnesium in the buffer from 1 to 10 mM increased the rate of energy dissipation, and the sample no longer showed the striped structure.

The appearance of the microtubular stripes shows a striking resemblance to the formation of layers in the dissipative space structure of the Belousov–Zhabotinski reaction[6]. Also, long rod-like polymer liquid crystals that are far from equilibrium owing to the application of an external magnetic field form a similar dissipative structure comprising alternating layers of different orientation[27]. Together, these observations strongly suggest that the microtubular state reported here is a dissipative spatial structure explicable in terms of irreversible nonlinear processes.

While this work was being refereed, two related articles have appeared. Mandelkow *et al.*[28] have demonstrated dissipative behaviour in a sample showing bulk oscillations of microtubule assembly and dissassembly. Depending on the conditions, dissipative systems can show either time-dependent or steady-state structures. The behaviour reported by Mandelkow seems to be of the time-dependent kind, whereas here a steady-state structure is achieved. Hitt *et al.*[29] have reported optical observations of a macroscopic microtubular structure similar to ours. They did not detect the periodic mutually orthogonal microtubule ordering from stripe to stripe, however, and offered no explanation of the striped structure. They realized that the microtubules were oriented by domains and explained the local orientational ordering in terms of packing considerations. But, as mentioned earlier, such considerations do not account for the striped structure nor its dependence on an energy source. Their samples were made in H_2O buffers, and therefore the dissipative structures are not restricted to D_2O-based buffers and could arise *in vivo*.

Mitotic spindles, which are constituted largely of microtubules show a pattern of bright and dark regions when viewed through a polarizing microscope[24,30], consistent with the spindles containing orthogonally oriented microtubular domains. The stripes reported here show substriations similar to those of mitotic spindles[24]. This aspect of our observations will be discussed elsewhere, but suggests that microtubular dissipative structures may form during mitosis. □

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Differential interaction of chiral β-particles with enantiomers

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VESTER[1] and Ulbricht[2] invoked the parity-violating weak interaction as the cause of biological homochirality. They postulated that a stereoselective synthesis or decay of chiral molecules could be induced by the left circularly polarized bremsstrahlung; this small effect would then be amplified to full homochirality[3–5]. Despite much effort during the past thirty years, experimental results have been inconclusive and contradictory[6–12]. The aim of the study described here was more modest; we did not want to demonstrate differential synthesis or the decay of enantiomers bombarded by β-particles. Rather, using pulse-height spectroscopy, we examined the Cerenkov radiation emitted as β-particles pass through liquid chiral enantiomers. Our results show that helical electrons do distinguish between molecules of opposite chirality, and complement results reported in earlier publications in which differential decay[6,13], positron annihilation[14,15], scattering[16,17] or other interactions[18–20] were observed.

The expectation of differential interaction of helical β-particles with enantiomers is based on an analogy with the theory of optical rotary dispersion (ORD) and circular dichroism (CD). There, the refractive indices and molar absorption coefficients of enantiomeric pairs are slightly different for left and right circularly polarized photons owing to the opposite helical potential field provided by the helically distributed static charges of the enantiomers. Thus the helical photons propagate with different velocities (ORD) and are absorbed to different extents (CD). Similarly, the left-helical β-electrons, with spin and momentum anti-parallel, should propagate with different velocities in the two enantiomers. The energy exchange between naturally chiral β-particles and the oppositely chiral molecules should also be different. One of us proposed[12] that the differential effect could be measured by pulse-height spectroscopy of the Cerenkov radiation, because this also depends on the refractive indices of the medium. We used enantiomers that are liquid at room temperature to avoid problems associated with dilution in achiral solvents.

Cerenkov radiation is the bluish light emitted as high-energy charged particles pass through a transparent medium at a speed *v* that is greater than the velocity of light *c* in that medium[21,22]. The intensity of Cerenkov radiation, a pulse, is proportional to the energy of the charged particle; a plot of counts per minute (c.p.m.) against channel number yields a pulse-height spectrum[23]. Provided the magnitude of the effect is large enough, the scintillation counter should detect an energy shift in the pulse-height spectra of β-particles in oppositely chiral molecules, because of the different propagation velocities. But the magnitude of the effect cannot be predicted because the Bethe equation[24,25], which relates the stopping power of the medium to the energy of the β-particles, contains neither the chiral form factor for the medium, nor the spin momentum coupling of the β-particles. Preliminary results[12] were promising; the shift was observed, but the resolution of the pulse-height spectra was unsatisfactory. Here we report high-resolution pulse-height spectra which allow us to calculate the energy exchange between β-particles and enantiomers, the slowing time of the β-particles and the stopping powers of the enantiomers.

The resolved enantiomers (R)-(−)-and (S)-(+)-2-phenylbutyric acid (PBA) are liquid at room temperature. Optical rotation in different samples (chiral purity 99+%, Aldrich Chemical Co. and Norse Laboratories) varied between $[\alpha]_D = -88.0$ to -93.0 in case of R-PBA, and $[\alpha]_D = +89.2$ to $+92.0$ for S-PBA. The refractive index and density were 1.5156 and 1.055 g cm^{-3}, respectively. Absorption, luminescence, infrared and CD spectra indicated that there was no contamination; spectra were essentially identical for the R- and S-enantiomers. ^{32}P (carrier free, H_3PO_4; ICN and New England Nuclear) emits a continuum of β-particles with mean and maximum energies of 695 keV and 1,710 keV, respectively; the corresponding left-handed polarizations (v/c) are 0.906 and 0.973, as calculated from the refractive index. The Cerenkov low-energy threshold for PBA is 169 keV, corresponding to 0.690 polarization, thus ~90% of the β-particles will emit Cerenkov radiation. For details of the method, see ref. 12.

Although it was tacitly assumed that the β-particles are emitted from the ^{32}P nuclides with the same energy in both enantiomers, it cannot be decided *a priori* that the chiral environment does not influence the energy of the β-particles. R- and S-PBA were therefore bombarded with external ^{32}P β-particles—instead of dissolving the $H_3{}^{32}PO_4$ in the liquid enantiomers, it was applied to and dried on a brass rod, covered with a 0.016-mm-thick sheet of polyvinyl chloride, and positioned in the vial ~1 cm above the liquids.

Figure 1*a* shows that the pulse-height spectra of R- and S-PBA with dissolved ^{32}P β-particles are not superimposed—the peak of R-PBA appears 13.5 channels toward lower energy than does the peak of S-PBA, an apparent energy difference of 41.4 keV. The longitudinally polarized β-particles therefore distinguish between the mirror-image molecules. The differential spectrum (Fig. 1*b*) more clearly demonstrates the shift with the scale of the ordinate magnified. The effect is larger than the 2σ uncertainty. When, the enantiomers are bombarded by β-particles from an external source, however, there was a noticeable decrease in the resolution of the differential spectrum (Fig. 1*c*). The peaks of R- and S-PBA differ by only six channels and the standard deviation is large. Therefore an influence from the chiral environment, cannot be entirely discounted. These results may, however, reflect the effects of an inherently different geometry of samples bombarded from an external source. The β-particles from an external source collide first with PVC and air molecules, and thus partially lose both velocity and polarization before reaching the PBA molecules. The density of interaction between electrons from the external source and PBA is also greater toward the surface of the enantiomer and interference phenomena may occur. These differences may also explain why previous investigations, in which molecules were bombarded from an external source[7–9], did not detect a differential interaction between chiral molecules and β-particles.

The differential spectra produced by polarized ^{32}P β-particles were compared with those produced by non-polarized high-energy electrons. Pellets of ^{137}Cs (Amersham Co.) were enclosed in a β-particle-absorbing shield (made of 2-mm-thick layers of copper and lead) and positioned inside the scintillation vial

TABLE 1 Change in peak position with ^{32}P flux density

Count rate (c.p.m.)				Channel of peak			
R-PBA	Racemic PBA	S-PBA	Water	R-PBA	Racemic PBA	S-PBA	Water
1,343,261 ± 78,585	1,400,098 ± 21,056	1,311,497 ± 47,140	1,050,570 ± 28,572	288.2 ± 1.6	299.7 ± 3.8	299.1 ± 5.9	262.4 ± 0.2
791,274 ± 41,052	844,664 ± 978	825,198 ± 26,199	623,518 ± 16,661	283.6 ± 3.0	303.1 ± 2.9	295.9 ± 6.1	260.3 ± 0.4
315,592 ± 6,886	331,845 ± 645	322,743 ± 10,328	244,566 ± 6,358	280.2 ± 3.8	300.6 ± 3.4	291.6 ± 6.3	257.9 ± 0.6
298,821 ± 9,199	293,848 ± 1,910	296,601 ± 27,906	220,296 ± 6,649	284.9 ± 1.6	290.5 ± 2.2	297.2 ± 1.2	252.4 ± 1.3
176,552 ± 6,500	178,466 ± 702	176,855 ± 16,431	132,913 ± 3,613	283.0 ± 1.4	300.5 ± 2.9	296.5 ± 1.7	256.4 ± 1.1
68,770 ± 2,654	70,149 ± 362	72,605 ± 6,510	52,193 ± 1,269	279.5 ± 1.1	301.5 ± 4.3	295.6 ± 1.8	255.7 ± 2.0

For the Beckman LS 3801 scintillation counter and phenylbutyric acid used in this study, the following equation relates Cerenkov energy (keV) with the channel number, N: $E = \exp[(N + 638.6)/151.7]$. The equation is not accurate for energies ⩽350 keV owing to the inefficiency of photomultiplier tube at low light intensities. Thus, the pulse-height spectrum does not overlap with the energy spectrum of the β-radiation. Fortunately, the problem is unimportant for our purpose because the peak of the spectrum and the maximum energy of the β-particles from ^{32}P lie far above the 350-keV threshold. The following efficiencies were calculated; R-PBA, 65.3 ± 1.6; Racemic-PBA, 66.5 ± 1.4, S-PBA, 66.3 ± 2.3; water, 49.8 ± 1.8. The efficiency of water is in agreement with earlier observations[23]. The unusually high efficiencies of PBA is due to its high refractive index. The channel of the peaks was determined from a best-fit polynomial to 15 points centred around the maximum of each spectrum; a minimum of four spectra were evaluated for each group. The correlation coefficient was >0.98 for each curve.

* Racemic mixtures were prepared immediately before bombardment but many crystallized during the experiments.

~1 cm above the PBA. The γ-particles eject non-polarized Compton electrons from the molecules and these high-energy particles then emit Cerenkov radiation. The differential spectrum of the non-polarized Compton electrons (Fig. 1*d*) is a straight line, as expected.

Despite the statistical confidence, we conducted two further control experiments to discount the possibility that the observed effect was contributed to or caused by systematic errors associated with the instrument, or by undetected contamination in the PBA. The main concern about the scintillation counter was saturation of the photomultiplier tubes and overspill of signals from one channel into another. Although experiments with different amounts of ^{32}P demonstrated that high count rates cause a shift of the pulse-height spectra (Table 1), the difference between R- and S-PBA is clearly observable at all count rates, regardless of the shift caused by high count rate, and varies between 11 and 16 channels. The saturation edge is located at ~200,000 total c.p.m. Table 1 also includes the peak location of racemic mixtures. It was not surprising that the peak of the pulse-height spectra of the racemic mixture is not located between the resolved compounds; the physical parameters of racemic mixtures are different from those of the enantiomers.

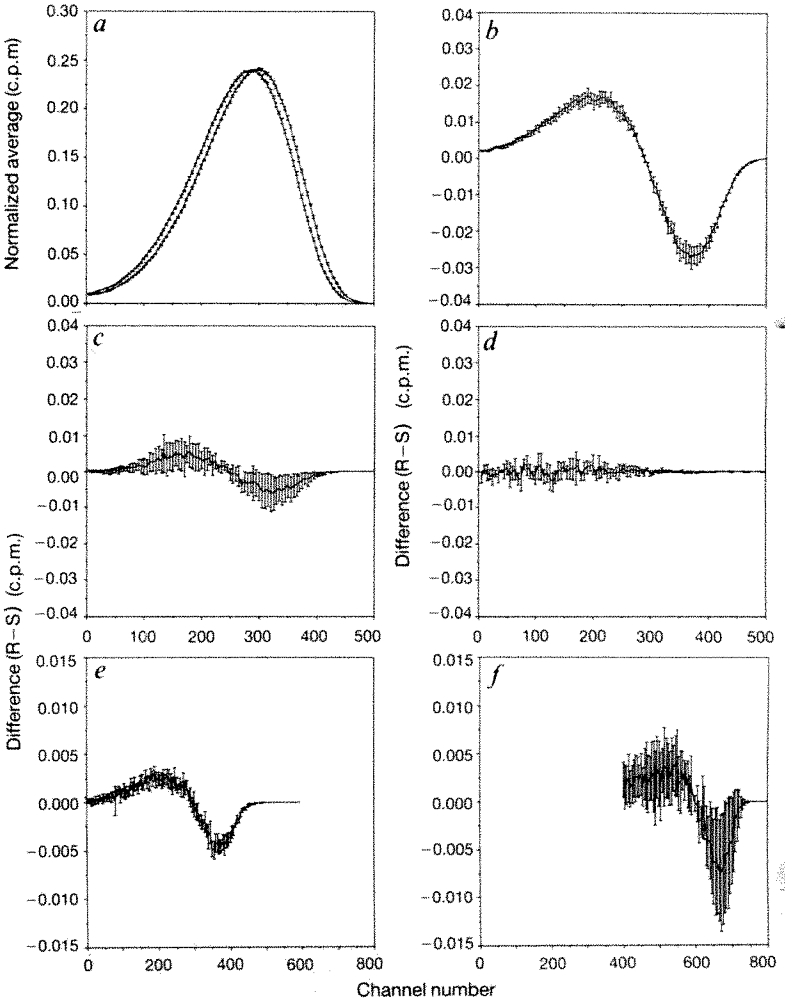

FIG. 1 *a*, Cerenkov pulse-height spectrum of ^{32}P dissolved in R- or S-PBA. Differential spectra (*b–f*) are generated by subtraction of the count rate in each channel of S from those of R. Differential spectra of *b*, R- and S-PBA shown in *a*; *c*, external ^{32}P and *d*, Compton electrons from ^{137}Cs. Scintillation differential spectra for *e*, ^{35}S and *f*, ^{32}P. Scintillation solutes were PPO and POPOP, see ref. 12 for method. A smaller amplitude for these processes may reflect the multiple achiral energy transfer processes of the liquid scintillation measurement. The first part of the ^{32}P spectra was removed to minimize the Cerenkov effect recorded in low-energy channels and concomitant irradiation from β-particles from unavoidable ^{33}P contamination. The latter may contribute to the high standard deviation on the ^{32}P differential scintillation spectrum. Note the different scales on the ordinate of the differential spectra. All spectra represent the average of four PBA samples measured in random order; the instrument was calibrated before each measurement. Saturation of photomultiplier and overspill between channels were avoided by assuring low count rates; samples of <200,000 total c.p.m. were used. All spectra were taken in five-channel increments and normalized to the total count rate. Error bars represent the standard deviation for each point. This set of experiments was repeated eight times with different batches of enantiomers and ^{32}P, a total of 32 spectra. All measurements yielded consistent results.

TABLE 2 Cerenkov interaction of β-particles with R- and S-PBA

^{32}P β-interaction	R-PBA	S-PBA
Apparent energy of peak of pulse-height spectrum (keV)	434.50 ± 4.11	475.50 ± 5.20
Collisional stopping power (MeV cm^2 g^{-1})	2.041 ± 0.050	1.997 ± 0.005
Distance travelled (mm)	1.268 ± 0.017	1.441 ± 0.022
Slowing down time (ps)	7.939 ± 0.080	8.731 ± 0.100

Data obtained by solving the Bethe equation[24,25].

Resolved PBA is liquid at room temperature, whereas the freshly made racemic mixture solidifies to soft crystals within a few hours or days. Owing to crystallization, the stopping power of racemic mixtures should not be the median of R- and S-PBA.

To examine the influence of possible contaminants, R- and S-PBA were contaminated with iron ethylenediamine tetraacetic acid; 0.05 μmol is the critical concentration at which the peak of the pulse-height spectra is shifted ~7 channels toward lower energy with a concomitant 17% decrease in Cerenkov efficiency. This small concentration is easily detected both spectrophotometrically and visually. As mentioned earlier, absorption spectroscopy did not indicate the presence of colour contamination, nor were efficiency differences between R- and S-PBA observed. A second type of contaminant could be any spectrophotometrically undetectable organic substance such as methanol, ethanol and ethylene glycol, introduced during preparation of the liquid compound. The peak of the pulse-height spectra was at channel 269 to 270 for these compounds. Therefore, a considerable amount of contaminant would be necessary to cause an 11-16 channel shift as observed between R- and S-PBA. In fact, dilution experiments showed that 10% contamination with methanol did not cause a significant change in the pulse-height spectra of either the R- or S-PBA. Such a large contamination is, of course, extremely unlikely; according to the high-performance liquid chromatography data supplied by the manufacturers, contamination of the resolved compounds is <0.1%.

Table 2 summarizes our calculations. The stopping power of R-PBA for β-particles is significantly higher than that of S-PBA. Consequently, the chiral electrons travel a longer distance and their slowing time is greater in S-PBA than in R-PBA. The analogy between the different refractive indices for circularly polarized light in enantiomers and the differential emission of Cerenkov radiation by helical β-particles is thus supported. Excitation and ionization are the predominant processes by which β-particles lose energy. The Kronig-Kramer[26,27] theorem relates the refractive index to excitation: from the refractive-index-dependent ORD spectrum the excitation-dependent differential absorption spectrum can be calculated. The extent of the postulated analogy was therefore investigated by the differential scintillation spectrum of β-particles from ^{35}S (carrier free, H_2SO_4, ICN), the energies of which are below the Cerenkov threshold (mean 49, and maximum 167 keV; $v/c = 0.41$). The scintillation spectrum in Fig. 1*e* clearly shows a small but significant differential interaction for processes dominated by excitation. The scintillation differential spectrum for ^{32}P is presented for comparison (Fig. 1*f*).

The Sokolov-Loskutov[28] equation addresses the contribution of spin to Cerenkov radiation in achiral media. For the optical properties of PBA and the assumption that the refractive index varies little in the blue-violet region, the calculated spin effect is ~1 part in 10^{14} of the refractive-index-dependent emission, an apparently negligible contribution.

The observations reported here complement those in references 14, 15, 17-20, but the mechanism of interaction of high-energy β-particles in liquid enantiomers and the low-energy 'artificially' polarized electrons in chiral gaseous targets[17], must be entirely different.

Finally, the conservation of energy and the cause of the apparent energy difference of β-particles implied by shift of the pulse-height spectra is not fully understood. If the chiral environment influences the energy of the emitted β-particles, then the origin of the shift is easily explained. But if the β-particles are emitted with the same energy in each enantiomer, then the apparently 'missing' energy described by the shift reflects a difference in the distribution of the energy loss of the electron. In the S enantiomer, a larger fraction of the dissipation energy would appear as Cerenkov radiation and excitation, and a smaller fraction as translational, rotational and vibrational motion of the molecules; for the R enantiomer, the converse would occur. □

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Evidence for an early glacial maximum in the French Vosges during the last glacial cycle

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THE Last Glacial Maximum is observed to have occurred between 18,000 and 20,000 years ago throughout northern Europe. Here we present evidence for the surprising result that in the region of the French Vosges, the regional ice sheet advanced during the early part of the last glacial cycle, and this expansion was considerably larger than that which occurred during the Last Glacial Maximum. From these results, we conclude that the glacial maximum may not have occurred at the same time worldwide, contrary to what has usually been assumed[1].

Three glaciations successively built up the Linexert moraine complex, the group of Ecromagny terminal moraines and the Servance terminal moraines[2] (Fig. 1). The ice originated in the eastern Vosges highlands (1,424 m above sea level (a.s.l.)), descending west and southwest through several valleys, the largest of which is the Moselle. During the first two advances the ice overflowed the northern crest of the Ecromagny Plateau (750-800 m a.s.l.), reaching down to elevations of 300 and 500 m a.s.l. During the Servance glacial advance, however, the ices did not reach the plateau but entered the Ognon valley through the pass at Col des Croix (Fig. 1) which lies at 683 m a.s.l.

All three moraine groups are complex features and may represent several recessions and readvances. The petrographic composition of glacial deposits reflects the bedrock over which the ice advanced—near Ecromagny and Linexert the moraines, tills and related outwash deposits contain a high percentage of detritus from the metamorphic rocks outcropping in the Ecromagny Plateau, whereas the outwash and tills connected with the Servance moraines are dominated by granitic detritus (Fig. 1).

Numerous depressions, which today are marshes and peat bogs, were formed by glacier erosion. Many of them are closed basins without surface outflow, surrounded by higher ground built in part by the bedrock and in part by the tills. Here we discuss cores from two of them—the Grande Pile bog, which lies between the Linexert and Ecromagny moraine lines, and the Cusenier peat bog, which lies on the Plateau behind the innermost Ecromagny moraine (Fig. 1).

Multiple bores drilled in the Grande Pile bog recovered ~18 m of silty clay, peat and gyttja, overlying the glaciolacustrine tills of the Linexert glaciation. In Fig. 2 we show the principal features of the Grande Pile core in a composite section from Woillard[3,4] to which we have added the ^{14}C data of Woillard and Mook[5] and our petrographic information. The pollen diagram[3,4] proves that the Linexert glacial advance is older than the last interglacial (Lure) and that the Ecromagny advance, which did not overrun the Grande Pile site, is younger. Three ash layers are present in the Grande Pile core. The Ternuay ash, the source of which is unknown, is older than 70,000 years and its position relative to the pollen zones places it at the end of the Lure interglacial, which is correlated with the oxygen isotope stage 5e (ref. 4). The Melay ash, probably from the Eifel Massif, is between 20,120 and 28,880 years old and the pollen analysis places it in the period of cooling that led to the Last Glacial Maximum. It is correlated with the lower part of oxygen stage 2. The youngest ash is correlated with the Laacher See Tuff 5-Final, which is about 10,800 years old[6].

The Grande Pile peat bog has always been isolated from the surface hydrographic network and the only contribution of detritic minerals to the predominantly organogenic sediments comes from the local hillwash and from the wind-blown dust or volcanic ash.

We identified the heavy minerals using a petrographic microscope, and verified them using Gandolfi camera X-ray analysis. The heavy minerals are derived either from the granites, metamorphic rocks or Triassic sediments outcropping in the Vosges (Figs 1 and 2). Detritic calcite accompanies the metamorphic group. In the organic muds of Lure interglacial the content of heavy minerals was too low to allow interpretation. The oldest detrital Linexert sediments in Grande Pile originated mostly from sedimentary and metamorphic bedrock. In the Mélisey I zone the granitic assemblage indicates transport from the braided plains of rivers flowing through the granites. In Mélisey II the granitic assemblage is succeeded by the metamorphic one,

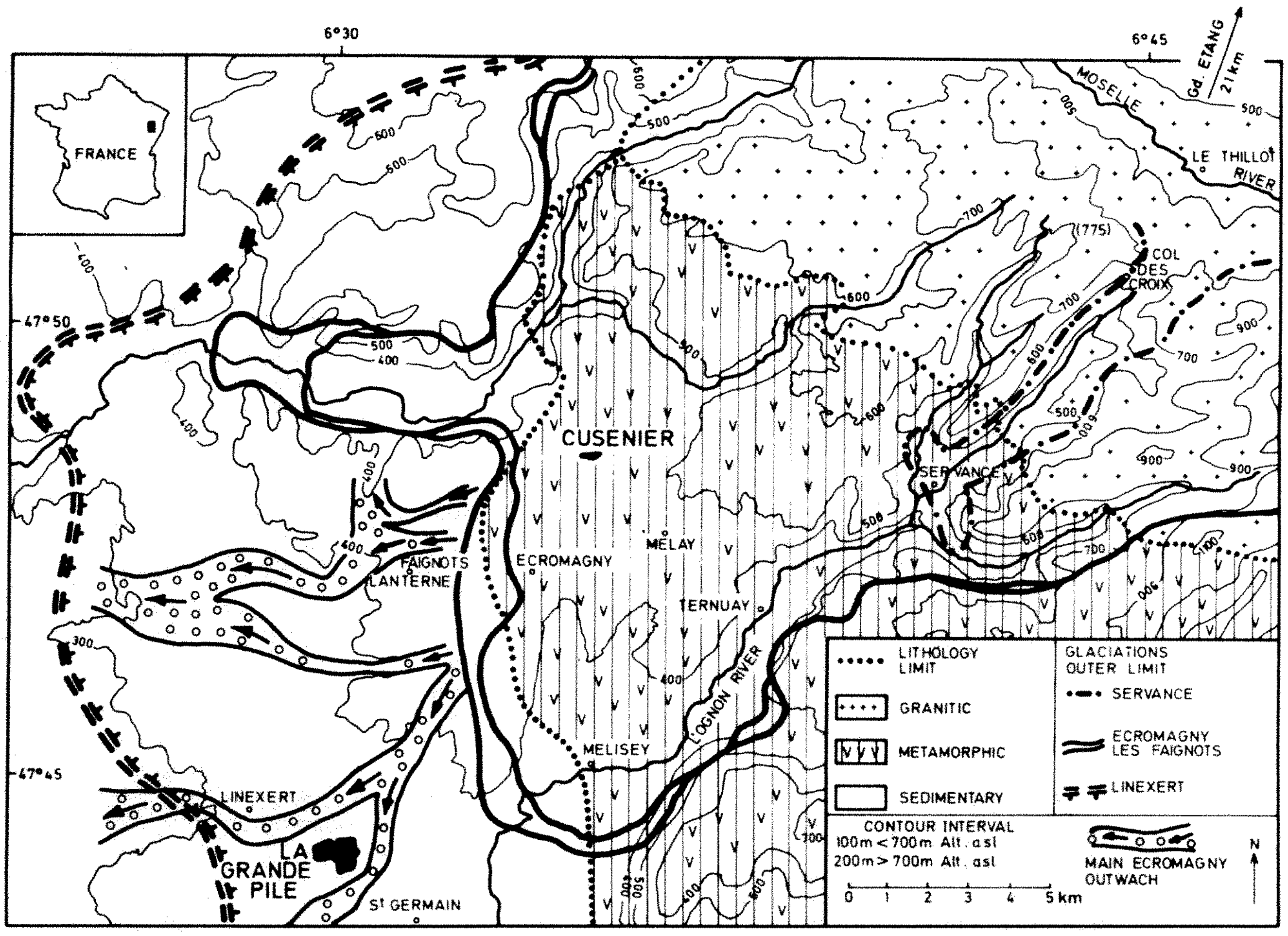

FIG. 1 Glaciations on the Ecromagny plateau.

indicative of sources on the Ecromagny Plateau. Between the St-Germain II and Pile interstadial, (~70–50 kyr ago), the source of the aeolian dust is the granites and sedimentary rocks. A sharp increase in the metamorphic content appears after the Pile interstadial at ~45–50 kyr, which we interpret as Ecromagny glacier outwash in the vicinity of the Grande Pile peat bog. During the Grand Bois interstadial at ~30 kyr there was a second shift of the mineralogical composition of aeolian dust back to granitic, indicating the absence of an ice sheet on the plateau.

The Cusenier peat bog is situated in a depression left by the retreating Ecromagny glacier. Like Grande Pile, it is isolated from any sources of detrital minerals other than local slope wash and aeolian transport. The core obtained in Cusenier bog in 1987 (Fig. 2) revealed peat, organic mud, lacustrine clays and silts overlying the lodgement and waterlain tills of the Ecromagny glacial advance[7]. The pollen diagram corresponds closely to the upper part of the Grande Pile core. It covers the end of the Grand Bois interstadial, the peak glacial tundra correlating with oxygen isotope stage 2 and the Holocene. The Melay ash is at 6.25 m depth and the Laacher See ash at 4.70 m. The Cusenier core shows that the glacier advance over the Ecromagny Plateau is older than the Melay ash and therefore older than 20,000 years. The mineralogical record of the Grande Pile core indicates that the outwash from the Ecromagny glacier became abundant at about the Pile interstadial, which suggests that the glacier advanced to its outermost moraines and retreated during the interval between the Pile and the Grand Bois interstadials (50–30 kyr).

The glaciers of the Servance stage did not build up high enough to cross the crest of the Ecromagny Plateau and only the upper part of Ognon valley was invaded by the ice, through the Col des Croix pass at 683 m a.s.l. (Fig. 1). The Grand Etang depression located inside the Servance moraine group, but outside the area shown in Fig. 1, has a till at the bottom followed by proglacial laminated clays, organic mud and finally peat. Only the Laacher See ash is present and the pollen diagram correlates with the uppermost part of the Last Glacial Maximum zone, the Late Glacial and the Holocene (Fig. 2).

Figure 2 also shows the smectite content of the clay mineral fraction. The smectite occurs in the Grande Pile mainly in the upper part of the Linexert sediments, in Mélisey I, in the Ognon complex and in the sediments between Grand-Bois and the Late Glacial. This last smectite event is also present in the Cusenier sediment. The smectites are well crystallized in the upper parts of both Linexert and the Last Glacial Maximum deposits, and in the Grand Etang sediments from the upper part of the Servance glaciation. The transition to the Late Glacial deposits is marked by a progressive decrease and finally complete disappearance of smectites and, in Grand Etang, by a progressive decrease of crystallinity.

The work of Blaise[8] shows that there is close correlation between well crystallized smectite and a dry cold climate. It has even been suggested that not only is the preservation of smectite favoured by the cold climate but that it can also be formed under such conditions[8,9]. We therefore associate the units containing smectite with periods of increased dryness. Because they appear to postdate the maximum expansion of the ice sheets in this area, the disappearance of the ice was probably due not only to warmer conditions but also to a decrease in precipitation. On the other hand the cold zones with absent smectite correspond to ice advances, which according to the palynological data were not the coldest ones in the core[4]. It therefore seems that the ice sheet grew in response to a large snow supply in a humid but not extremely cold climate.

Our evidence shows that a large body of piedmont ice existed in the southern Vosges at least once after the Lure interglacial and before the Last Glacial Maximum. The lower age limit is set by the St-Germain I temperate phase, the vegetation of which is incompatible with the presence of ice in the Vosges. The upper age, between 20 and 30 kyr, is recorded in the Cusenier core, which indicates that the ice sheet melted before the Melay ash and probably during the Grand Bois interstadial. The onset of the ice build-up is more difficult to fix—only when the ice sheet reached the Ecromagny Plateau could the fine detritus of metamorphic rocks registered in the Grande Pile cores since ~50 kyr be released into the local streams.

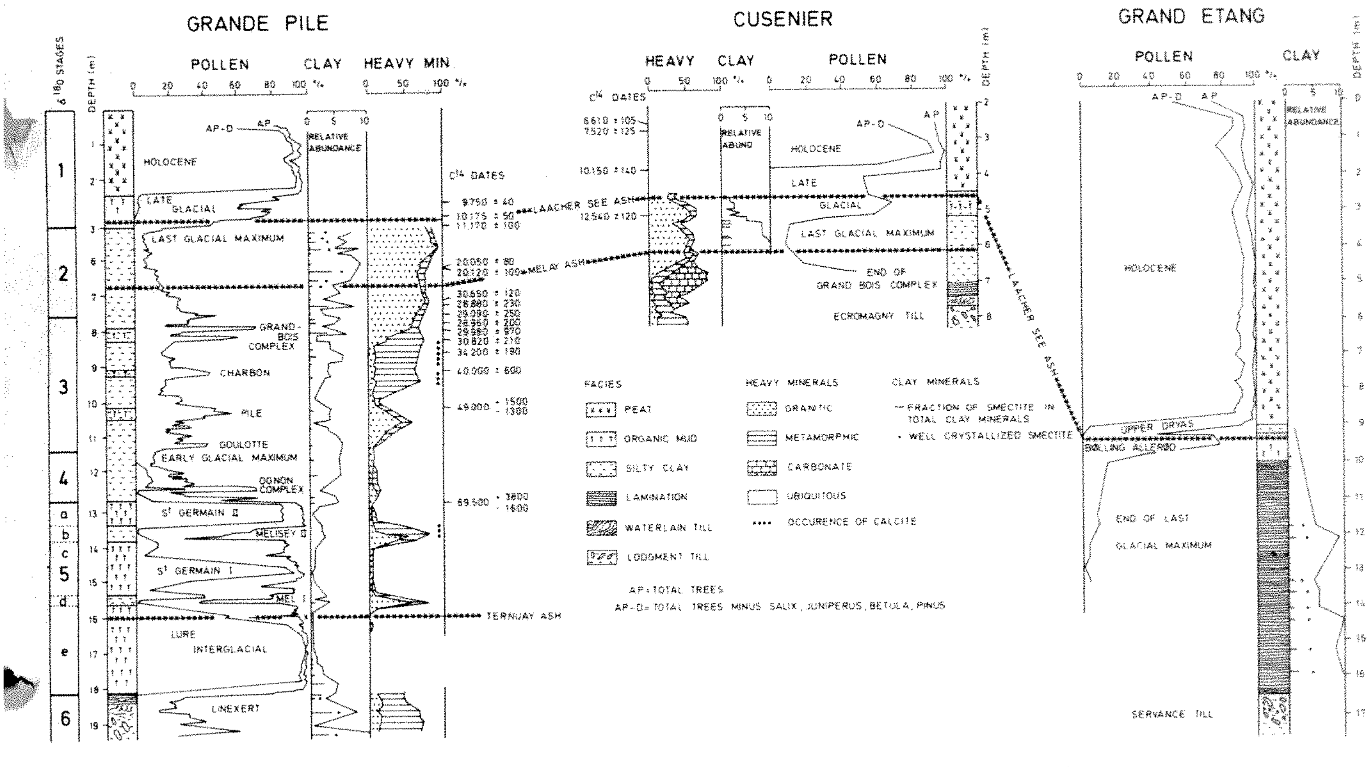

FIG. 2 Data from composite cores taken from bogs at Grande Pile, Cusenier and Grand Etang.

In our opinion two models are possible. According to the first, the ice build-up on the plateau started after St-Germain II, during the Early Glacial Maximum in oxygen isotope stage 4, and retreated during the Goulotte warming, at the start of oxygen isotope stage 3 some 60 kyr ago. During its retreat it left behind bare surfaces covered by tills rich in detritus of metamorphic rocks and these became the sources of the aeolian silt that reached the Grande Pile bog. According to this model the ice sheet present during oxygen isotope stage 4 would not have supplied any outwash and metamorphic detritus to the Grande Pile, which seems rather unlikely.

In the second model, which we favour, no ice was present on the Ecromagny Plateau during oxygen isotope stage 4—only some time after 50 kyr would the ice overflow the crest of the Ecromagny Plateau and, in two pulses, leave behind the twin Ecromagny moraines. This model bears some resemblance to the ice advances of Ontario lobe where tills of the Cherry Tree stadial separate the 48-kyr-old Port Talbot interstadial from the 23 to 32-kyr-old Plum Point interstadial[10].

The degree to which the mineral composition of Mélisey I and Mélisey II silts indicate regional ice advances remains unclear. Whereas Mélisey I and the lower part of Mélisey II show a granitic source, the upper part of Mélisey II contains heavy minerals of metamorphic origin as well as detritic limestone. It is possible that the ice had reached the Ecromagny Plateau crest in Mélisey II (correlated with oxygen isotope stage 5b) because 80% of the metamorphic detritus is too coarse to be moved very far by wind. In addition, the absence of smectite suggests a large humidity supply, compatible with the growth phase of the glacier.

Whatever the details, a large ice advance must have reached the area between 50 and 30 kyr, because the sedimentation of Cusenier is well dated (Fig. 2), with continuous evolution from till to a complete and progressive glacier retreat sequence. The ice volume and extent were considerably larger than that during the Last Glacial Maximum.

The presence of a large ice mass at relatively low latitudes and elevations in western Europe long before the Last Glacial Maximum at 20 kyr favours models that link the mechanism of global cooling to changes in the snow supply and to the major reorganization of oceanic and atmospheric circulation in the North Atlantic[11–13]. The position of the polar front in the North Atlantic fluctuated around 45° N during the Late Pleistocene[14,15] and the largest range of surface air temperature also occurred in this belt[16,17]. The front of the southern Vosges is close to this latitude, at ~48° N. The sequence of events in the sediments points to large variations in the moisture supply. It seems that the coldest phases were dry, resulting in a smaller glacier, and that growth of the ice sheet was accompanied by a large snow supply in a somewhat warmer climate. Our results also indicate that the glacial maximum may not have occurred at the same time around the world. ☐

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U–Th ages obtained by mass spectrometry in corals from Barbados: sea level during the past 130,000 years

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THE study of the sea level record during the last glacial cycle has primarily proceeded indirectly by means of oxygen isotope measurements on foraminifera from deep-sea sediments[1,2]. The direct approach of dating sea level indicators stagnated during the past decade, mainly because the samples required to complete our knowledge of the past glaciations are below the present-day sea level[3–7]. Using the ^{14}C ages of *Acropora palmata* samples collected by drilling offshore the island of Barbados, Fairbanks[8] presented the first detailed chronology for the last deglaciation. This radiocarbon chronology is limited to the past 30 kyr because of the short half-life of ^{14}C (5,730 yr); we must therefore rely on other dating methods to obtain information for the whole last glacial cycle. During the past four years ^{230}Th–^{234}U dating of corals by thermal-ionization mass spectrometry has been shown to be significantly more precise and accurate than the classical α-counting method[9–14]. We have used this technique to measure U–Th ages in coral samples from the Barbados collection and from subaerially exposed outcrops (see also ref. 15). Here we present results bearing on the sea level record for the past 130,000 years; we conclude that the last deglaciation started 3,000 years earlier than previously thought and confirm that there were two surges in melt water[8] at about 11 kyr and 14 kyr BP (before present).

The technique used for U–Th measurements is described elsewhere[16]. The activities and dates are listed in ref. 15 and Table 1. The agreement of our U–Th ages with the calibrated ^{14}C ages is excellent for the past 10 kyr and we have shown that the two geochronometers can be reconciled between 10 and 30 kyr if we take into account the variation of the atmospheric ^{14}C/C ratio inferred from the changes of the strength of Earth's magnetic dipole[15].

We measured the sample RGF1-17-4 in duplicate at the accelerator mass spectrometry facility of Gif-sur-Yvette and determined that it was older than 48 kyr, the ^{14}C dating limit[16]. This is in agreement with its U–Th age determined by mass spectrometry, 70.8 ± 0.6 kyr (Table 1).

The initial ^{234}U/^{238}U ratios are calculated from the measured ^{234}U/^{238}U, using the ages obtained from the ^{230}Th–^{234}U chronometer[10]. All corals younger than 70 kyr fall in the range of the modern sea water (1.14–1.15, ref. 9). This confirms that the Barbados corals grew in open sea water and that the U–Th system has not been strongly altered after deposition. By contrast, all corals older than 70 kyr have initial ratios between 1.15 and 1.165 (some samples have ratios as high as 1.2), in good agreement with the mass spectrometric results of Edwards[10] and Chen and Wasserburg[17] and with the recent α-counting results by Ku *et al.*[18]. The interpretation of ^{234}U/^{238}U ratios in the older corals from Barbados and other Caribbean islands will be discussed in detail elsewhere (B.H., E.B., R.G.F. and A. Zindler, manuscript in preparation). One possible explanation is that all corals have been contaminated by exogenous uranium early in their evolution and that all ^{230}Th ages are essentially correct. An alternative explanation is that the ^{234}U/^{238}U ratio of sea

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TABLE 1 U–Th isotope data

Sample	Depth* (m)	^{238}U (p.p.m.)	[^{230}Th]/[^{234}U]†	^{234}U/^{238}U	(^{234}U/^{238}U)$_{init}$‡	Th age (yr BP)
RGF12-30-2#1	−78	3.22±0.02	0.2458±0.0016	1.1255±0.0067	1.1368±0.0074	30,470±240
RGF12-30-2#2		3.46±0.02	0.2428±0.0014	1.1314±0.0062	1.1431±0.0068	30,040±210
RGF1-17-4	−57	3.62±0.02	0.4840±0.0027	1.1294±0.0060	1.1582±0.0076	70,820±600
RGF8-27-1#1	−46	3.56±0.02	0.5254±0.0030	1.1517±0.0063	1.1900±0.0083	79,400±700
RGF8-27-1#2		3.58±0.01	0.5180±0.0032	1.1501±0.0061	1.1871±0.0080	77,800±800
FS-13	18	3.34±0.02	0.5634±0.0031	1.1293±0.0065	1.1661±0.0087	88,200±800
AFZ-2	6	3.07±0.01	0.6113±0.0038	1.1145±0.0064	1.1522±0.0090	100,500±1,100
AFM3#1	55	3.04±0.02	0.6944±0.0046	1.1077±0.0072	1.1536±0.0110	125,100±1,700
AFM3#2	55	3.12±0.01	0.6941±0.0026	1.1065±0.0037	1.1518±0.0057	125,100±1,000

U–Th results obtained on *A. palmata* samples from Barbados (see ref. 15 for samples younger than 30 kyr). Duplicates were measured on different pieces of the sample. All errors are quoted at the 2σ level. All samples contain negligible amounts of calcite (X-ray diffraction measurement).

* Depth or altitude of recovery relative to present sea level.

† Measured atomic ratios were converted to activity rates using U–Th half-lives given in ref. 10.

‡ Initial ^{234}U/^{238}U ratios were obtained using Th ages to correct measured isotopic ratios.

water changed by ~1.5% since 100,000 yr BP. Therefore, all the corals with initial ratios between 1.15 and 1.165 have not been strongly altered and their ^{230}Th ages probably represent true ages of formation. By contrast, the corals with initial ^{234}U/^{238}U ratio higher than 1.17 have been contaminated by exogenous uranium and their ^{230}Th ages represent lower bounds on their ages of formation.

Figure 1 presents our sea level estimates obtained by U–Th dating, the normalized δ^{18}O curves determined by Shackleton[19] and Labeyrie *et al.*[20] and the 65° N summer insolation curve[21]. In both cases the δ^{18}O chronologies have been orbitally tuned[19,20].

We have measured U–Th ages of coral samples corresponding to the last interglacial period from different Caribbean islands. These corals are not at the same altitude on the different islands because of the tectonic uplift that affects the Caribbean subduction zone. On the island of Curacao, which is thought to be relatively stable, corals from the last interglacial period are found at ~5–10 m above the present sea level[22] in accordance with U–Th ages measured on corals collected in stable areas[23]. By contrast, on Haiti[24] and Barbados[25–27] those corals are found at altitudes between 40 and 60 m above the present sea level (on Barbados the uplift rates are even different in the southern and eastern parts of the island[27]). By assuming that the sea level of the last interglacial period was 7 m above the present level, we have recalculated all the results obtained on Barbados corals using a linear tectonic uplift correction[27]. The potential error on the sea-level reconstruction presented in Fig. 1 is smaller for younger ages and for the last deglaciation the uplift correction is practically negligible, of the order of the inherent error due to the habitat range of *A. palmata.*

Our high-precision results for the last interglacial period[15] show that all the corals from this sea-level highstand are restricted to a short period between 120 and 130 kyr in agreement with previous data[11,12]. They are in disagreement with the conclusion by Winograd *et al.*[28] that the last interglacial period took place at ~140–150 kyr, which contradicts the Milankovitch theory (B.H., E.B., R.G.F. and A. Zindler, manuscript in preparation).

We now compare our U–Th age estimates obtained for coral samples corresponding to the last glacial period. For periods beyond the ^{14}C timescale (>30 kyr BP) there were previously only mass spectrometric results for two samples[11]. Here we present five new samples which correspond to the interstadial periods of isotope stage 5, isotope stage 4 (sea-level low stand) and stage 3.

For the Barbados II terrace (Ventnor terrace in the Matthews[27] morphostratigraphic classification; isotope stage 5c) we measured an age of ~101 kyr (AFZ2) in agreement with the δ^{18}O chronologies and the mean age measured by α-counting (~105 kyr, refs. 25, 26; sample AFZ1 was dated by α-counting at 104±8 kyr, ref. 26).

This age differs significantly from the results obtained by Edwards[10] on sample FT-50 (112.0±1.0, 111.8±1.3, 112.3±1.1 kyr) which would put this sea-level highstand at the time of an insolation minimum[21]. This sample was dated by α-counting[18] at 103.6±4 kyr, in disagreement with the results by Edwards[10]. This may be because sample FT50 is heterogenous because of alteration. Indeed, both thermal-ionization mass spectrometry and α-counting results[10,18] show that this sample has an anomalously high initial ratio ^{234}U/^{238}U of ~1.17–1.18.

Sample FS13 corresponds to Barbados I terrace (Worthing terrace; ref. 27; isotope stage 5a) and has been dated at ~88 kyr in excellent agreement with sample OC-51 dated by Edwards (87.5±0.6, 87.9±0.7 kyr, ref. 10; sample FS3 was also dated by α-counting at 84±8 kyr; ref. 26). These ages are, however, slightly older than the mean of the dates measured by α-counting

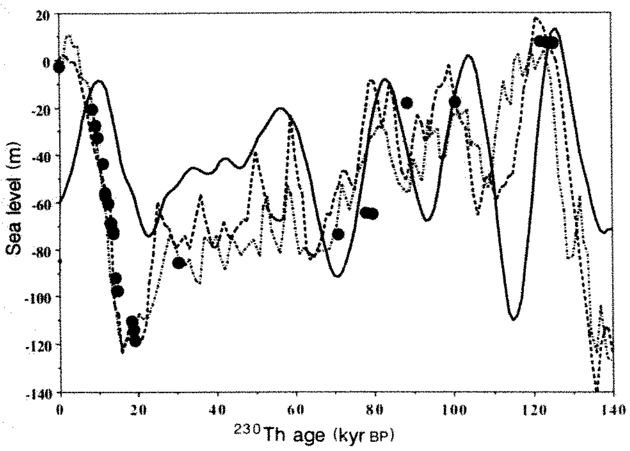

FIG. 1 *A. palmata* palaeodepth plotted against Th–U ages measured by mass spectrometry. To reconstruct this sea-level curve we applied linear corrections for uplift by normalizing the last interglacial high stands to 7 metres above the present sea level (see text). Barbados data are listed in Table 1 and ref. 15, data for samples C1 and C4 from Haiti are given in ref. 15. Dashed-dotted line, normalized δ^{18}O curve determined by Shackleton[20]; dashed line, normalized δ^{18}O curve determined by Labeyrie *et al.*[19]; solid line: 65° N summer insolation[21]. (δ^{18}O(‰)=[(^{18}O/^{16}O)$_{sample}$/(^{18}O/^{16}O)$_{standard}$]-1, where standard is PDB.)

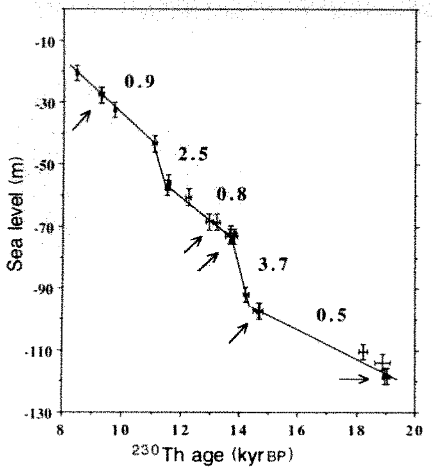

FIG. 2 Sea level during the last deglaciation. All samples are *Acropora palmata*. The U-Th age errors are quoted at the 2σ level. The samples marked by arrows correspond to duplicate analyses of the same coral sample by U-Th mass spectrometry. All duplicates are in agreement within error. A constant error of ±2.5 m has been assumed for the sea level. The numbers correspond to the melting rates (metres per century) calculated from slopes of the linear regressions.

on Barbados I (closer to 82 kyr, refs 18, 25, 26), which was used in the $\delta^{18}O$ tuning (Fig. 1). The mass spectrometric U-Th data suggest an earlier age for isotope stage 5a and therefore a smaller time lag between the climate response and the orbital forcing.

Samples RGF1-17-4 and RGF8-27-1 were collected below discontinuities characterized by erosion and dissolution features typical of a prolonged sub-aerial exposure[8]. These samples were dated at 70 kyr and 79 kyr and can be considered as contemporaneous with the early part of isotope stage 4 in rough agreement with the $\delta^{18}O$ chronologies.

Sample RGF12-30-2 was also collected below a sub-aerial exposure. This critical sample indicate a sea level of −85 m at 30 kyr and gives a new independent constraint on the sea level during isotope stage 3. Our data differ significantly from the only other available estimate, based on classical U-Th and ^{14}C dating of a coral sample from the Huon Peninsula in New Guinea (−40 m at ~30 kyr, ref. 29). The result obtained in Barbados and New Guinea could be reconciled by a rapid decrease in the sea level of ~40 m in <6 kyr at ~35 kyr (the 2σ error of the previous U-Th estimate is 5 kyr). This is supported by the reconstructions based on benthic $\delta^{18}O$ curve (Fig. 1) which exhibit high-frequency structures during isotope stage 3.

Figure 2 focuses on the coral record of the last deglaciation dated by U-Th. It can be seen that this abrupt climate event started before 19-18 Th kyr and was characterized by two large jumps dated at ~13.5 kyr and 11 kyr, called melt water pulses IA and IB[8] by Fairbanks. The first rise corresponds to a dramatic rate of sea level change of ~3.7 metres per century, followed by a decrease down to <1 metre per century and then by a second abrupt change of ~2.5 metres per century (Fig. 2). Those rates were already quantified by Fairbanks[8] using the ^{14}C chronology. The better precision and accuracy given by the U-Th chronology confirm that the rates of the meltwater pulses were higher than 10^6 km^3 of continental ice per century.

These new results have several important implications for palaeoclimate studies: (1) The fact that the two abrupt sea level changes exist in both ^{14}C and U-Th timescales demonstrates that the two jumps in the sea level record[8] and benthic $\delta^{18}O$ curves[30] are not artefacts of the ^{14}C timescale. (2) These results also suggest that the time lag between orbital forcing and ice-sheet response was overestimated when it was solely based on the ^{14}C chronology. The maximum value is of the order of a few kiloyears. Our results will help in choosing the climate time lag to refine the SPECMAP timescale[31] and in calculating the conversion factor between $\delta^{18}O$ and sea level changes[6]. (3) Large discrepancies exist between data on the local sea level obtained by the ^{14}C methods and those determined using geophysical models that calculate the response of a layered viscoelastic Earth to surface load forcing[32,33]. To explain the ^{14}C-dated sea level estimates and the recent tide-gauge data, very large Barents Sea ice sheet[32] and/or significant melting of Antarctica[33] has been invoked. Our data demonstrate that the last deglaciation started 3,000 years earlier than previously thought and this helps to explain the disagreement between observations and models (W. R. Peltier, manuscript in preparation).

Our mass spectrometric U-Th data provide a high-precision absolute chronology of the last glacial cycle. The Barbados sea-level curve has been augmented using data from submerged samples, enabling a close comparison with other chronologies inferred for the last glacial cycle[18,19,31,34-37]. In particular, the sea level was below −70 m during isotope stage 4 and below −80 m for the end of stage 3. The deepest *A. palmata* sample is dated at ~19 kyr and corresponds to a sea level of −118 m for the Last Glacial Maximum. As demonstrated by Fairbanks[8], the last deglaciation is characterized by two steps which are now dated by the U-Th method at ~11 kyr and 14 kyr. These two steps are the likely consequences of two dramatic meltwater surges which exceeded the rate of 10^6 km^3 of continental ice per century. □

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Three-liquid immiscibility and the origin of carbonatites

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THE relationship of rock types involved in carbonatite complexes has provoked much debate[1–4]. One favoured theory of origin involves the immiscible separation of carbonate-and silicate-rich liquids to produce the bimodal distribution of rock types typical of many complexes. Previous investigations of liquid immiscibility at crustal pressures in both natural[5,6] and synthetic[3] systems have demonstrated that immiscibility can produce much of the chemical diversity (in both major[3,5,6] and trace elements[7]) of this group of rocks; however, arguments persist regarding the relative importance and genetic relationship of alkali-rich and alkali-poor carbonatites[1,4,8–10]. Here we report the first results of new experiments at upper-mantle pressures, which show unexpected features indicating the existence of three immiscible liquids, one silicate- and two carbonate-rich, with surprising density relationships. These two carbonate liquids and the associated vapour could account for many carbonatite characteristics, while avoiding some problems encountered in deriving magmas of variable alkali content from a single carbonatite parent.

The photographs in Fig. 1 show selected areas of vertical sections through five different charges that have been rapidly quenched from 1,225 °C and 15 kbar. The majority of the experiments are in the system SiO_2–Al_2O_3–CaO–Na_2O–CO_2 although minor constituents have been added in some runs. The composition of starting mixtures and the resulting phases are listed in Table 1 and plotted in Fig. 2. The charges generally consist of a silicate bead (diameter equal to the capsule width, ~5 mm) situated towards the base of the charge, with a mixed carbonate above and a single carbonate below. Vesicles are always present, demonstrating the existence of a vapour phase. With certain bulk compositions a few silicate phenocrysts may also be present.

We interpret these charges to represent three immiscible liquids for the following reasons. All three phases can be seen in spherical or droplet form displaying a range of sizes, in some cases coalescing to form larger areas. These spheres are at least one or two orders of magnitude larger than the maximum sphere size (2 μm) produced by metastable quench immiscibility[11] and boundaries are sharp (<1 μm). The composition of each phase seems to have been homogeneous before the quench and entrained vesicles support a liquid interpretation. Very similar phase compositions are produced from a wide range of starting mixtures so long as the bulk compositions plot within the three-liquid field of Fig. 2. This consistency is independent of the run duration or the textural relationship of the phases.

The presence of an almost pure Ca carbonate liquid at 1,225 °C requires further attention as the melting point of calcite at 15 kbar should be >1,500 °C (ref. 12). The Ca carbonate in these charges displays the morphology of a liquid, following the complex shape of the capsule wall and exhibiting a curved meniscus against other liquid phases. Dumbbell-shaped Ca carbonate inclusions have also been observed in the silicate liquid. Ca carbonate liquids do not quench to glass, even at the rapid

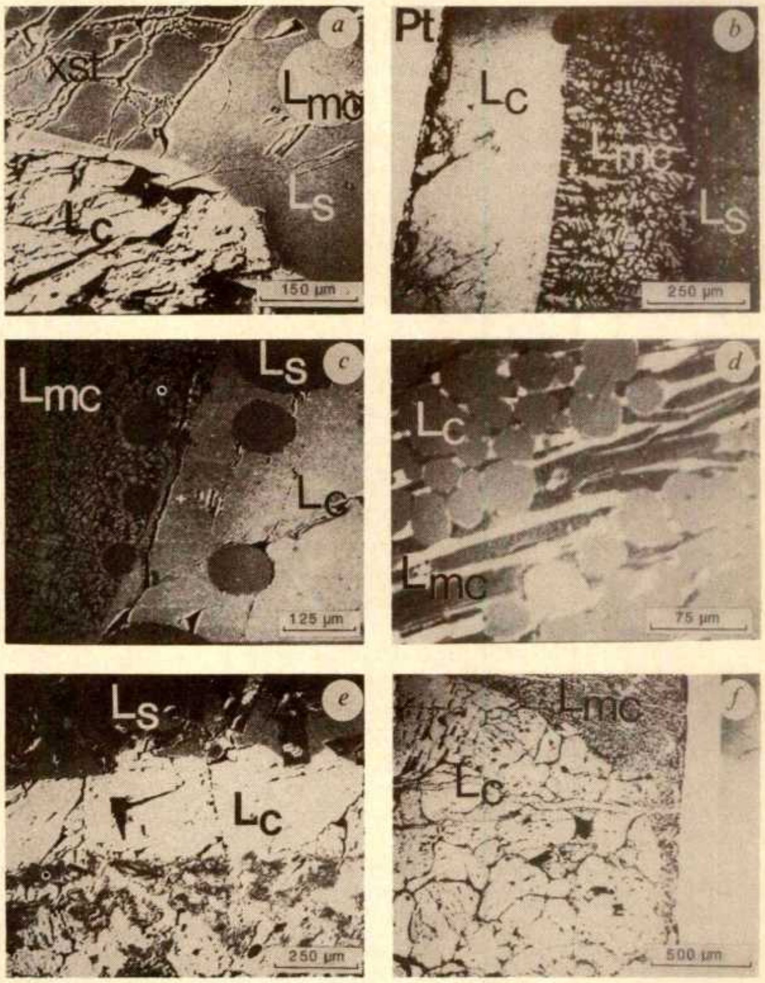


FIG. 1 *a–e*, Back-scattered electron (BSE) images taken on Cameca microprobe. *f*, Reflected-light photomicrograph. All images are orientated with the top of the charge uppermost; *a* and *e* are different areas of the same charge. *a*, Three liquids together. The lightest area (L_c), with many triangular cleavage pits, is almost pure Ca carbonate. This area shares a smooth curved boundary with a silicate glass (L_s) which contains a large crystal (xst) and a sphere of mixed Na–Ca carbonate (L_{mc}). At higher magnification this sphere is seen to consist of an intergrown, two-carbonate quench texture typical of a (homogeneous) mixed-carbonate liquid[3]. *b*, Lightest area on the left is the platinum capsule wall (Pt), this is in contact with an area of quenched Ca carbonate melt (L_c). The next area inwards shows the typical dendritic quench texture of the mixed Na-Ca carbonate (L_{mc}). The remaining area (L_s) is silicate glass. Vesicles (black) can be seen on the boundary between L_c and L_{mc} and in L_s towards the top of the photograph. *c*, Boundary between dendritic mixed carbonate (L_{mc}) and the lighter Ca carbonate (L_c) quenched liquids with spheres of silicate glass (L_s). *d*, Products of a run with BaO added to the starting composition (see Table 1, RB127). The medium grey, coalescing spheres (L_c) are Ca carbonate. The background lamellae (L_{mc}) represent another typical quench texture of a mixed Na–Ca carbonate liquid. *e*, Arrangement of the phases in the base of the charge. The darker area (L_s) is the bottom of the silicate bead and contains numerous phenocrysts. The area between the bead and the base of the capsule (bottom of photograph), is occupied by Ca carbonate liquid (L_c). The patches in L_c are clusters of small crystals of the same composition as the larger phenocrysts in the silicate (L_s) above. *f*, An area of Ca carbonate (L_c) in the base of a charge sharing a sharp curved meniscus with a mixed Na–Ca carbonate liquid (L_{mc}). The Ca carbonate contains a few small (50 μm) spheres of silicate glass which are difficult to see in reflected light. Note the curvilinear nature of the grain boundaries which form the mosaic pattern of the Ca carbonate area.

TABLE 1 Run data

Run	RB104	RB110	RB113	RB126	RB127	RB130*	RB132	RB133
Run time (h)	24	30	31	5	18	18	24	20
Bulk composition								
SiO_2	4.20	20.61	22.33	20.61	20.88	22.33	21.61	20.93
Al_2O_3	1.20	10.34	8.83	10.34	8.26	8.83	8.63	8.43
CaO	45.00	31.64	31.64	31.64	29.58	31.64	32.18	31.61
Na_2O	9.60	8.81	8.81	8.81	8.24	8.81	7.56	6.88
K_2O	—	—	—	—	—	—	1.36	—
BaO	—	—	—	—	5.05	—	—	—
MgO	—	—	—	—	—	—	—	2.39
CO_2	40.00	28.60	28.39	28.60	27.99	28.39	28.66	29.76
Silicate liquid (L_s)								
SiO_2	48.31	50.44	49.20	48.00	46.88	50.17	48.20	38.58
Al_2O_3	19.70	20.66	18.13	21.79	19.73	20.00	20.10	16.67
CaO	19.11	13.77	20.58	15.94	17.34	16.56	15.20	25.52
Na_2O	10.88	12.13	10.09	10.27	10.01	11.27	9.10	6.45
K_2O	—	—	—	—	—	—	2.10	—
BaO	—	—	—	—	3.68	—	—	—
MgO	—	—	—	—	—	—	—	2.61
SF†	2.00	3.00	2.00	4.00	2.36	2.00	5.30	10.16
Mixed carbonate liquid (L_{mc})								
SiO_2	3.42	2.88	3.17	2.77	3.30	2.94	4.20	4.41
Al_2O_3	0.30	0.24	0.31	0.35	0.36	0.24	0.60	0.59
CaO	46.06	44.28	39.42	45.00	42.81	45.72	46.90	45.25
Na_2O	11.28	12.60	9.10	10.68	10.50	11.10	8.70	8.20
K_2O	—	—	—	—	—	—	1.00	—
BaO	—	—	—	—	6.41	—	—	—
MgO	—	—	—	—	—	—	—	1.92
SF	38.94	40.00	48.00	41.20	36.62	40.00	38.60	39.60
Ca-carbonate liquid (L_c)								
SiO_2	0.28	0.20	0.30	0.40	0.41	0.25	0.28	0.89
Al_2O_3	—	—	—	—	—	—	—	0.14
CaO	55.44	56.80	55.50	54.70	50.27	56.00	56.00	54.28
Na_2O	0.28	—	0.20	0.30	1.24	0.30	0.50	0.37
K_2O	—	—	—	—	—	—	—	—
BaO	—	—	—	—	4.21	—	—	—
MgO	—	—	—	—	—	—	—	0.39
SF	44.00	43.00	44.00	44.60	43.87	43.45	43.22	44.00

All runs at 1,225 °C and 15 kbar. Compositions are in weight per cent.

* The starting mix of RB130 included 20 wt% of calcite cleavage rhombs.

† These analyses were obtained using an electron microprobe, which cannot analyse volatile components. SF indicates the shortfall for the phase and in the case of the carbonates this is equivalent to the expected stoichiometric amount of CO_2. The volatile component producing the shortfall in the silicate phase has been analysed using infrared and preliminary result show that it is composed mainly of CO_2 but some water is also present.

quench rate of these experiments (1,225 °C to 700 °C in 8 s), and the outline is filled by either a single crystal or a mosaic of crystals each with optical continuity and sets of parallel cleavage (see Fig. 1*f*). The small individual globules of Ca carbonate typical of runs in the low-alkali two-liquid field (see Fig. 3*b*, *c*) are also shown to be crystalline by X-ray diffractometry.

To try to demonstrate that Ca carbonate is molten under these conditions, calcite cleavage rhombs were arranged throughout a capsule, replacing most of the usual $CaCO_3$ powder in the starting mix. These rhombs were not present in the resulting charge, which had a single area of Ca carbonate in the base and the usual phase compositions (see Table 1). The low liquidus temperature of the Ca carbonate may be due to the presence of water in the charges. Although every attempt was made to ensure that the starting materials were dry, infrared analysis of the silicate glass bead indicated the presence of water, possibly formed as a result of hydrogen diffusion through the platinum capsule wall during the run[13].

Another unexpected feature of the Ca carbonate liquid is its position towards the base of the capsule. Although there have been no direct measurements of Ca carbonate liquid density, an estimate of 2.2 g cm^{-3} has been made by comparison with Na carbonate[14]. These experiments suggest that at 15-kbar Ca carbonate liquid is not only more dense than the Ca–Na-carbonate liquid, but also of similar or higher density to a conjugate nephelinite-type silicate liquid. The contrast in physical behaviour between alkali-rich and alkali-free carbonates, is further demonstrated (see Fig. 3) in the two-liquid fields, above and below the three-liquid stability field (see Fig. 2).

All runs show evidence of a vapour phase, but it has not been possible to obtain quantitative analyses. Mass balance considerations, however, indicate that a substantial amount of Na partitions into the vapour, and in similar experiments at lower pressure Koster van Groos and Wyllie[15] identified a vapour containing "much" dissolved Na, which they suggested could represent an agent of fenitization.

The results of these synthetic experiments provide a new flexibility to carbonatite genesis modelling. The addition of further components to this simple system is important in evaluating the relevance of any model to natural rocks and we have included the results of additions of minor quantities of MgO, K_2O and BaO in Table 1 to give some indication of how some of these components may behave. A comparison of the results from natural[5] and synthetic[3] systems, however, shows the value of the simple analogy in interpreting the more complex situation.

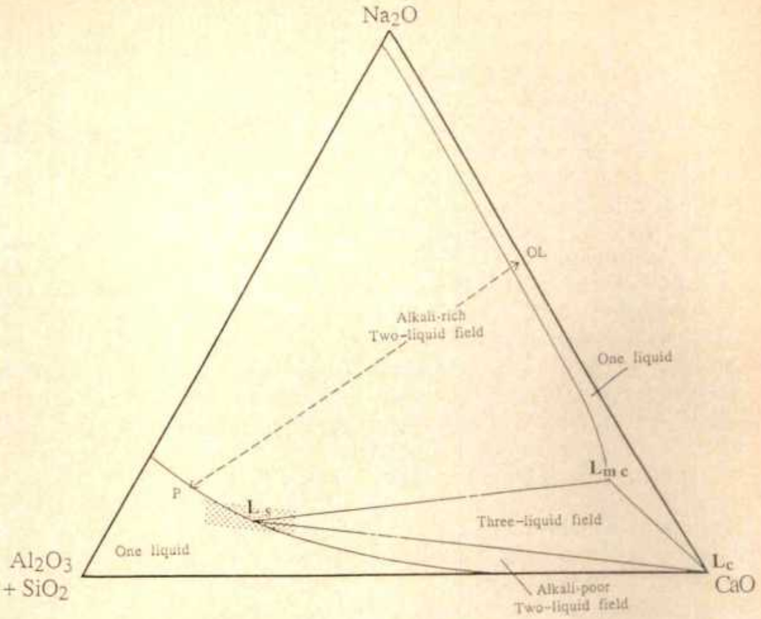
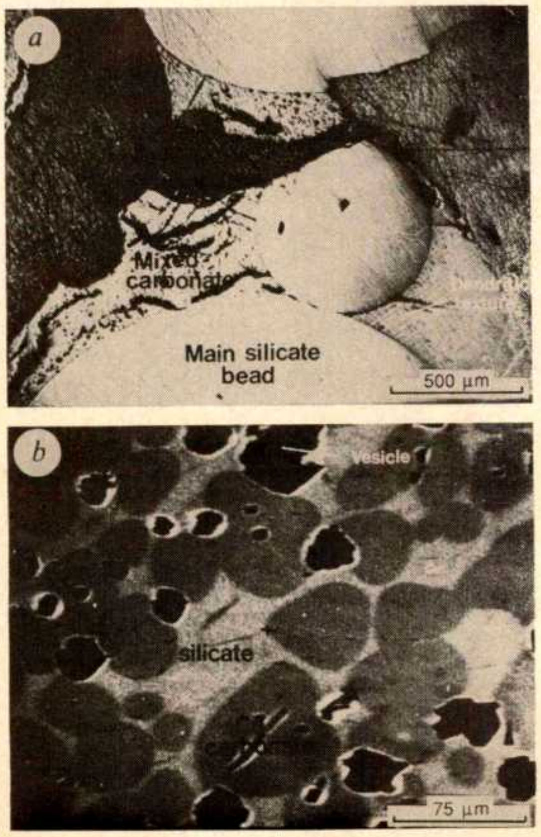


FIG. 2 The compositions of the three liquids at 1,225 °C and 15 kbar are plotted on a pseudo-ternary wt% diagram. To enable the representation of five components, SiO_2 and Al_2O_3 are combined in one corner and a projection is made from CO_2; carbonate liquids contain 44–60 wt% CO_2 and silicate liquids 2–5 wt%. Any starting composition within the three-liquid triangle will produce L_s, L_{mc} and L_c in variable quantities. These compositions appear reasonably constant despite a variety of SiO_2: Al_2O_3 ratios in the starting composition thereby justifying the combination of these 'network formers' in one corner. To enable natural rock compositions to be plotted on this diagram, K_2O is added to Na_2O, and FeO, Fe_2O_3 and MgO are added to CaO (after ref. 5). The composition of an Oldoinyo Lengai natrocarbonatite (OL) is shown along with the experimental conjugate silicate liquid which corresponds to a phonolite (P). The dotted area is the range of typical nephelinites (and ijolite).

Although many conclusions can be drawn from these results, we draw attention to the following possibilities.

Immiscibility could be induced as a CO_2-saturated nephelinitic magma cooled to intersect the two- or three-liquid solvus (these experiments show one liquid at 1,325 °C and two at 1,250 °C), or by an increase in the CO_2 concentration of the liquid owing to fractionation of non-carbonate-bearing phases. LeBas[2] suggests that magma collects in a reservoir at the mantle/crust boundary, building up CO_2 by the precipitation of olivine and clinopyroxene. The parental magma would plot closer to L_s in Fig. 2 than the experimental starting compositions and the proportion of both carbonate liquids would consequently be much smaller.

If three liquids were produced then the low-density carbonate liquid corresponding to L_{mc} would separate first, perhaps reaching the surface to produce carbonatite lavas or lapilli tuffs. With the exception of Oldoinyo Lengai, all natural extrusive carbonatites are calcitic[4,16] (or dolomitic[17]), most containing calcite phenocrysts. Crystallization of these phenocrysts at atmospheric pressure from the observed melt composition is difficult to accept because of the problem of dissociation. One possible explanation is that the original lava had a substantially higher alkali content,



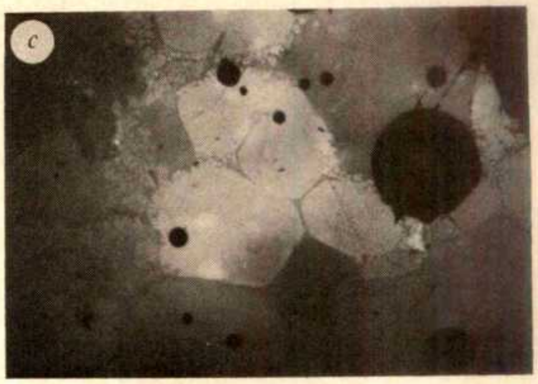


FIG. 3 *a*, Products of a run in the alkali-rich two-liquid field. This reflected-light photomicrograph shows horizontal sections through a main (and minor) silicate bead that is surrounded by alkali-rich carbonate quenched liquid with the typical dendritic texture, just visible at this magnification. The composition of the silicate glass is comparable to a phonolite and the carbonate is similar to the natrocarbonate of Oldeinyo Lengai. *b*, Runs in the alkali-poor two-liquid field contain numerous alkali-free carbonate globules surrounded by a silicate glass. This BSE image shows globules of Ca-carbonate which never separate into a single area even if left for four days although this immiscible texture is achieved in less than a few hours. *c*, Reflected-light photomicrograph of the charge from a run just below the three-liquid field. Small globules of Ca-carbonate are seen coalescing to form larger globules (of similar size to those in *b*), which appear to collect in the base of the capsule and deform to give curvilinear boundaries. This run was quenched after only 5 h and suggests that a texture similar to that in Fig. 1*f* could be produced eventually.

which was subsequently modified by meteoric water. The discovery of what appeared to be calcite pseudomorphs after primary nyerereite, seemed to support this conclusion[8,10]. This led LeBas[8] to suggest that a natrocarbonatite such as Oldoinyo Lengai (75% alkali carbonate) would be the parent of most carbonatites, the high alkali content escaping as fenitizing fluids to leave compositions capable of crystallizing primary calcite and nyerereite. But many workers dispute the inferred existence of nyerereite[16-18], and Gittins[4] has shown that only 15 wt% Na_2CO_3 is required in a calcitic carbonatite to allow calcite to crystallize at atmospheric pressure as long as >5 wt% fluorine is also present. Such a lava would correspond to L_{mc} in our study, with the small amount of alkalis being easily lost from the ground mass. The role of fluorine is important in these rocks and although the low liquidus of the Ca carbonate (L_c) in these experiments is attributed to accidental H_2O, a small amount of fluorine would produce a similar effect[4].

Models in which fenitizing fluids emanate from a natrocarbonatite, removing enough alkalis to produce the typical carbonatite composition, may be criticized on several points. As suggested above, the evidence for alkali-rich extrusive rocks has been seriously questioned[9,16], and there is no clear evidence from intrusive carbonatites for any intermediate compositions or for the large volume reductions and replacement features that would be produced by alteration of such rocks. Some compositionally zoned silicate minerals in intrusive carbonatites actually show an increase in alkalis from cores to rims[1]. The occasional absence of fenites around some carbonatites and a closer spatial association with the silicate rocks in some complexes[19,21] could be explained by a vapour phase associated with all the magmas rather than by a vapour emanating from one in particular (again, fluorine may be important).

The intrusion sequence with carbonatites cross-cutting earlier silicate rocks has led to criticism[1] of models involving immiscibility owing to the presumed low density of carbonate liquids. But the Ca carbonate seems to be the most dense of these liquids at 15 kbar and would therefore be intruded late in a complex, perhaps partially crystallized.

These preliminary experimental results show that three-liquid immiscible fractionation may be possible under upper-mantle conditions and indicate that a carbonate liquid may be more dense than a conjugate silicate liquid. Further investigation involving the addition of components such as MgO, FeO, TiO and in particular phosphorus and fluorine may help to evaluate further the relevance of this mechanism to carbonatite genesis. ☐

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Pteropod abduction as a chemical defence in a pelagic antarctic amphipod

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THIS study documents an example of an invertebrate that cannot defend itself chemically (an amphipod) increasing its chances of survival by capturing and carrying a species that can (a pteropod). Although chemical defences are found in a wide variety of marine invertebrates[1-4], few studies have established the extent to which these chemicals will deter predators[5-7], and even fewer have investigated how one organism might exploit another's chemical defence to protect itself[8-10]. These chemicals are usually ingested or sequestered in the host's tissue[8,9]. Several species indiscriminately decorate themselves with potentially toxic organisms or are passively fouled with chemically defended organisms[11,12]. These seem to be commensalisms or sometimes mutualisms. Our example has benefits and costs for the carrier, but only costs to the captive. The antarctic marine food web not only has a variety of chemically defended organisms[13-16], contrary to earlier predictions[17-19], but has at least one unusual symbiosis that makes use of noxiousness.

The pelagic hyperiid amphipod *Hyperiella dilatata* is one of the most common prey of the abundant, plankton-eating fish *Pagothenia borchgrevinki*[20,21]. Moreover, this amphipod is found in the stomach contents of at least five other common antarctic fish (*P. bernacchi, P. hansoni, Trematomus centronotus, T. nicole, Gymnodraco acuticeps*)[21,22]. *Hyperiella dilatata* and other members of the Hyperidae lack conspicuous morphological attributes such as spines, which might ward off fish predators[23]. But in McMurdo Sound, Antarctica, during the austral summer, we observed amphipods selectively carrying small individuals of the abundant pelagic pteropod *Clione limacina* (Table 1; Figs

TABLE 1 Frequency of *Hyperiella dilatata* carrying *Clione limacina* at two sites in McMurdo Sound, Antarctica

	No. amphipods with pteropods	No. amphipods without pteropods	% Amphipods with pteropods
Coastal site (9 m depth)			
Total	225	80	74 (range 61–81)
Offshore site (50 m depth)			
Total	93	1,394	6 (range 5–10)
Offshore site (7–10 m depth)			
Total	17	35	33(range 29–35)

Plankton nets (0.75-m) equipped with 505-μm mesh were deployed at depths of 9 and 50 m at two sites (shallow site near shore, 3 km northeast of Hut Point on Ross Island; deep site offshore, 1 km south of McMurdo Station). During November 8–27, 1989 the coastal site was sampled 4 times, whereas the offshore deep and shallow sites were sampled 6 and 2 times, respectively. Plankton nets were positioned horizontally and allowed to fish passively for 24 h. The numbers of paired and unpaired pteropods and amphipods were noted immediately. Ratios of amphipods to pteropods were roughly equivalent at both coastal and offshore sites (range, 1:1–1:3 amphipods to pteropods). Relative mean abundances of pteropods were 267 per 24 h of net deployment at the shallow site, and 32 and 653 per 24 h net deployment at the 7–10 m and 50 m offshore sites, respectively. Absolute abundances (number per 1,000 m^3) of *C. limacina* and *H. dilatata* near McMurdo Station are reported by Foster *et al.*[20].

TABLE 2 Fish acceptance/rejection of the pteropod *C. limacina* and the amphipod *H. dilatata* when carrying pteropods

	Clione	Cod pieces	Agar with *Clione*	Agar without *Clione*	*Hyperiella* with *Clione*	*Hyperiella* without *Clione*
Fish accept	0	23	0	20	2*	13
Fish reject	23	0	20	0	10	0
Fisher exact test (*P*)	<0.001		<0.001		<0.0001	

Living *C. limacina* (2–20 mm length) were offered individually to a group ($n=97$) of *P. borchgrevinki* (14–20 cm length). Preliminary observations revealed that a prey item presented to the group of fish caused a general feeding response with many individuals (>50%) attempting to feed. Each pteropod was paired with a similarly sized piece of flesh from the antarctic cod *Dissostichus mawsoni*. In 23 independent feeding trials, all cases resulted in multiple violent rejections of the pteropod from the mouths of at least 10 of the fish. All fish tissues were immediately ingested. A similar experiment was performed using standardized agar blocks (0.5×0.5×0.5 cm) containing a 1% dry weight concentration of fish meal. Experimental blocks were prepared with 1% fish meal and 10% (wet weight) whole homogenized *C. limacina*. In each of 20 trials, at least 10 different fish rejected agar blocks containing a 1% fish meal and pteropod homogenates, whereas fish rapidly ingested control agar blocks containing only 1% fish meal. Living individuals of *H. dilatata* and those carrying *C. limacina* were attached with a small amount of silicon grease to the end of a fine needle on a glass rod. Individual amphipods or amphipods carrying pteropods were rubbed lightly against the mouths of individual *P. borchgrevinki* ($n=17$ fish; 14–20 cm length) until taken into the mouth. The order of feeding trials was randomized. All amphipods without pteropods were ingested ($n=13$), but those carrying pteropods were violently spat out (8 with pteropod attached and 2 with pteropod separated from amphipod). In two other instances (*), the pteropod was separated from the amphipod in the mouth and only the pteropod ejected, indicating how important it is that the amphipod should tightly grasp the pteropod.

1 and 2*a*). Amphipods were observed *in situ* through holes drilled in the sea ice and after capture by plankton nets. In both cases, we observed individuals carrying pteropods. In several experiments we demonstrated that *C. limacina* is chemically defended against predation by *P. borchgrevinki* (Table 2). Field studies have shown that although *C. limacina* is abundant, it is not eaten by *P. borchgrevinki*[20]. The chemical nature of the distasteful compound is not known, but it may be either derived from the diet or synthesized *de novo* as in other shell-less marine gastropods[24].

In a laboratory experiment we investigated whether the pteropod-carrying behaviour of amphipods could provide a chemical defence against fish predation. Individual *H. dilatata* (controls) and *H. dilatata*–*C. limacina* pairs (amphipods were seen always to carry only one pteropod) were collected from 9 m below the sea ice and offered to individual *P. borchgrevinki* in a randomly determined sequence. We recorded the numbers of pteropod-paired and unpaired amphipods ingested or tasted and then rejected. Observations were also made on the condition of the rejected individuals.

Pagothenia borchgrevinki showed a significant ($P<0.0001$; Fisher exact test) rejection for *H. dilatata* carrying *C. limacina*. In all cases, amphipods that were not carrying pteropods were readily eaten, whereas almost all of those carrying pteropods were rejected (Table 2). Moreover, some fish seemed visually and/or chemically to detect amphipods carrying pteropods, frequently approaching them and then swimming away. Avoidance may be related in part to the orange coloration of both the amphipod and the pteropod, providing fish with a strong visual

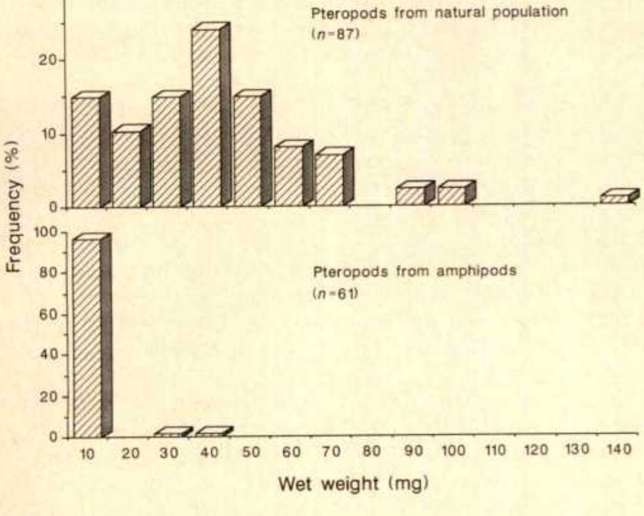

FIG. 1 Size frequency histogram showing the relative size (wet weights) of pteropods (*C. limacina*) sampled from the natural population, compared with individuals removed from the grasp of the amphipod *H. dilatata*. Pteropods removed from amphipods are significantly ($P<0.0001$; Mann Whitney *U*-test) smaller than those sampled from the natural population.

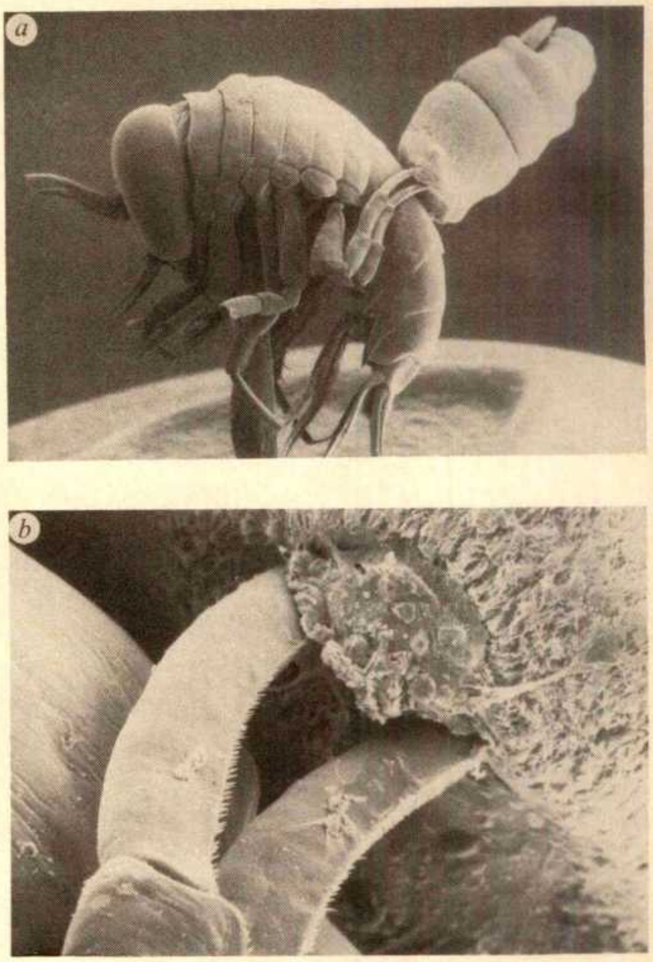

FIG. 2 *a*, Scanning electron micrograph showing the amphipod *H. dilatata* carrying the chemically defended pteropod *C. limacina*. Magnification is about 50×. *b*, Scanning electron micrograph showing the sixth and seventh pereiopods of the amphipod *H. dilatata* grasping the pteropod *C. limacina*. Magnification is roughly 500×.

cue. Although fish violently (with head shakes) ejected amphipod-carrying pteropods from their mouths, amphipod–pteropod pairs were generally not separated upon ejection, and in no instance were the amphipods or pteropods killed. Moreover, coupled individuals did not separate when handled or placed into a 10% solution of $MgCl_2$, reflecting the great tenacity with which the sixth and seventh pereiopods of *H. dilatata* grasp *C. limacina* (Fig. 2*b*). Amphipod–pteropod pairs observed in aquariums over a one-week period remained coupled. Uncoupled amphipods presented with swimming pteropods were observed to swim rapidly towards a pteropod and grasp the mantle posteriorally with the pereiopods. One set of the sixth and seventh pereiopods was then attached (Fig. 2*b*) and the retracted pteropod rotated dorsally. The opposing set of sixth and seventh pereiopods was then attached, securing the pteropod against the abdominal segments for carrying (Fig. 2*a*).

This study demonstrates that the pelagic hyperiid amphipod *H. dilatata* captures and carries the pteropod *C. limacina* to defend itself chemically from fish predation. Abducted pteropods tightly retract and therefore do not feed, although some uptake of dissolved organic material may occur across the body wall. Nevertheless, we never found dead pteropods. Although other hyperiid amphipods, including *H. dilatata*, commonly associate with scyphozoans[25–27], this is one of the few pteropod associations in this group. Trade-offs for the amphipod are involved in this symbiotic association. The benefits of pteropod carrying must be balanced against the increased energy expenditure necessary to counteract sinking when carrying a negatively buoyant mass. This is reflected in significantly reduced swimming speed. Swimming speeds (mean, ±s.d.) were 88.1 ± 22.1 ($n = 10$) and 53.5 ± 19.0 ($n = 10$) mm per second in individual and paired amphipods, respectively ($P < 0.01$; Student's *t*-test). Slower movements may impact both the ability of *H. dilatata* to feed and to avoid predators. Because abduction is facultative, amphipods may be able to adjust their defence to ambient predation risk. Whatever the cost–benefit relationship may be, the high incidence of pteropod carrying, particularly in shallow water where *P. borchgrevinki* is most abundant[20], indicates that this unusual behaviour is an important adaptation for a species that occurs together with a highly visual predator in the clear waters surrounding Antarctica. □

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Arachidonic acid metabolites as mediators of somatostatin-induced increase of neuronal M-current

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THE M-current (I_M) is a time- and voltage-dependent K^+ current that persists at slightly depolarized membrane potentials[1]. I_M is reduced by muscarinic cholinergic agonists and certain peptides, and is thought to be responsible in part for the slow and late slow excitatory postsynaptic potentials in sympathetic neurons[1–3]. Recently, we reported[4] that I_M in hippocampal neurons was also augmented by somatostatin-14 and -28 suggesting that two different receptors reciprocally regulate one neuronal channel type (ref. 4; see also ref. 5). Muscarinic effects on I_M may be mediated by various components of the phosphatidylinositol phosphate pathway[6,7]. We now report the involvement of a different second messenger pathway, that generated by phospholipase A_2[8–10], in the somatostatin-induced augmentation of I_M in hippocampal cells. This pathway generates arachidonic acid from which leukotrienes can be produced by lipoxygenases. We find that the I_M-augmenting effects of somatostatin are abolished by two substances that can inhibit phospholipase A_2[11], quinacrine and 4-bromophenacyl bromide, and that both arachidonic acid and leukotriene C_4 mimic the effects of somatostatin-14 on hippocampal pyramidal neurons *in vitro*. Arachidonic and somatostatin effects are blocked by a lipoxygenase inhibitor, implicating an arachidonic acid metabolite, perhaps a leukotriene, in the somatostatin effect.

We studied a total of 90 CA1 pyramidal neurons, with resting membrane potentials of -67 ± 0.5 mV and action potentials of 101 ± 1 mV (mean ± s.e.m.). A preliminary account of some of the data appears elsewhere[12]. We tested somatostatin-14 (SS14) and elements of the phospholipase A_2 (PLA_2) system on the I_M, using voltage clamp at holding potentials of −38 to −50 mV (mean −44 mV). Somatostatin-14 (1 μM) increased the amplitude of the I_M relaxation by 50–55% in 33 of 46 cells (Figs 1 and 2*a*, *e*). This was associated with an outward shift of baseline (holding) current relative to control and with a conductance increase (Fig. 1*a*, and 2*a*, *b*). The remaining 13 cells did not show an I_M response to somatostatin and were not studied further.

Quinacrine (8 μM; eight of eight cells) and 4-bromophenacylbromide (4-BPB, 10 μM; six of seven cells) abolished somatostatin-induced augmentations of I_M (Fig. 1*a*, *b*). Neither quinacrine (four cells) nor 4-BPB (four cells) alone in the superfusion medium had a measurable effect on I_M. Superfusion of arachidonic acid (120–150 μM) augmented I_M relaxations by 50–75% in 28 of 39 cells (Figs 1*c* and 3*a*), increased input conductance and caused an outward shift of baseline current at both resting and holding potentials around −45 mV. The remaining 11 cells were not responsive to arachidonic acid and were not studied further.

The Q-current seen in hippocampal pyramidal neurons[13] also produces an inward current relaxation with hyperpolarizing voltage steps, although only from hyperpolarized holding potentials (more negative than −70 mV). I_Q, but not I_M, can be inhibited by external Cs^+ (ref. 13), without an effect on the somatostatin-induced enhancement of I_M (ref. 4). As with somatostatin, arachidonic acid elicited I_M augmentation in three

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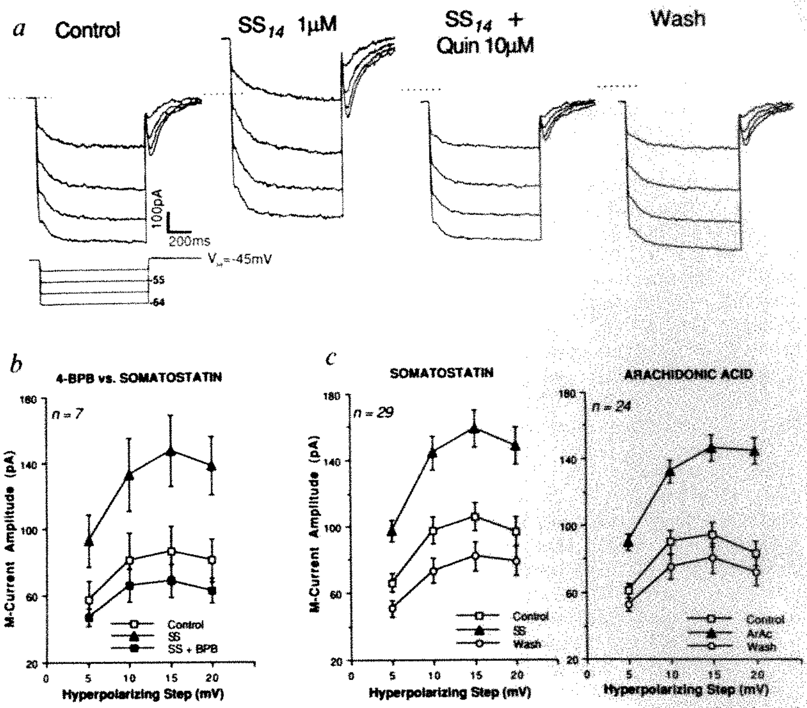


FIG. 1 *a, b,* PLA$_2$ inhibitors block effects of SS14. *a,* Digitized records from a pyramidal neuron of superimposed inward current responses to the voltage protocol shown below the left current traces, from a −45 mV holding potential. With this protocol, deactivation of the outward I_M persisting at −45 mV appears as a slow inward current 'sag' (relaxation) following the instantaneous (ohmic) inward current drop. Somatostatin increases the I_M relaxation amplitude, associated with an outward baseline (steady state) current (dashed line = control baseline current). Somatostatin also increases overall conductance at this holding potential (compare steady state current amplitudes). The PLA$_2$ inhibitor quinacrine (quin; 10 μM) significantly ($P < 0.01$) blocks all three effects of somatostatin. Time between each current panel in this and subsequent figures is 8–12 min. This and subsequent digitized ('raw') current figures (but not those used for curve fitting; see methods) were filtered digitally below 5 ms. *b,* 4-bromophenacyl bromide (4-BPB; 10 μM), another PLA$_2$ inhibitor (perfused together with SS14) also significantly ($P < 0.01$) blocked the somatostatin-induced I_M increase (averaged ±s.e.m. from seven cells). *c,* Arachidonic acid (ArAc; right panel) mimics the effects of SS14 (left panel) on I_M relaxation amplitude: average data obtained only from 29 of 42 (SS) or 24 of 34 (ArAc) cells clearly responding to these substances. Both somatostatin (1–1.5 μM) and arachidonic acid (120–150 μM) significantly ($P < 0.01$) augmented the I_M relaxation amplitude to about 160% of control in these responding cells. In this and most subsequent analyses, I_M measures were averaged from all cells tested with a given drug at each command and submitted to a 2-factor (drug × voltage step) analysis of variance for repeated measures (each voltage step), with Newman-Keuls post-hoc tests (allowing estimation of P values between any two groups) used to test specific drug effects.

METHODS. Rat transverse hippocampal slices (350–400 μm thick) were completely submerged and continuously superfused with warm (30–31 °C), gassed (95% O_2, 5% CO_2) artificial cerebrospinal fluid (ACSF). The composition of the ACSF was (mM): NaCl, 130; KCl, 3.5; NaH_2PO_4, 1.25; $MgSO_4 \cdot 7H_2O$, 1.5; $CaCl_2$ 2.0; $NaHCO_3$, 24; and glucose, 10. Intracellular and voltage clamp recording from CA1 pyramidal neurons was done with micropipettes filled with KCl (3 M; 50–80 MΩ). Drugs and peptides were added to the ACSF (with tetrodotoxin, 0.5–1 μM) in known concentrations. Arachidonic acid and leukotrienes were kept at −80 °C and used immediately after suspension; the lipids were dried under nitrogen and dissolved in the ACSF by sonication. NDGA, 4-BPB, and leukotrienes were superfused with 0.1% dimethylsulphoxide (DMSO), and arachidonic acid with 0.25% DMSO. DMSO alone in the superfusion medium had no significant effect on seven cells (data not shown). Indomethacin was superfused with 16 mM ethanol, which alone did not affect I_M. We used the single electrode voltage-clamp recording mode (Axoclamp preamplifier), with switching frequencies of 3–4 kHz. Electrode settling time and input capacitance neutralization at the headstage were monitored continuously. Current and voltage records were filtered at 0.3 kHz. We used holding potentials of −38 to −50 mV and hyperpolarizing commands of 5–20 mV and durations of 1 s. Current and voltage data were acquired by D/A sampling and acquisition software (P-Clamp: Axon Instruments); most often, −5, −10, −15 and −20 mV voltage commands were applied in sequence five times for subsequent averaging. Current measures thus were derived from averages of five sweeps at each of the four voltage commands. I_M amplitude was measured with software (Clampfit, Axon) that fitted exponential curves (1 exponent, R values = 0.85 − 0.99) to the I_M relaxation by two methods: (1) curve fitting to the peak of the initial (quasi-instantaneous) current that usually fell within 15–25 ms of step onset; or (2) exponential fitting through the peak with extrapolation back to command onset. These methods gave essentially equivalent results with respect to relative effects of drugs, although the extrapolated amplitude was obviously bigger (10–25%; average 20%) than amplitude at peak. For statistical purposes the first (peak) method was used. Baseline (holding) current and relative steady state conductance were also measured by the same software.

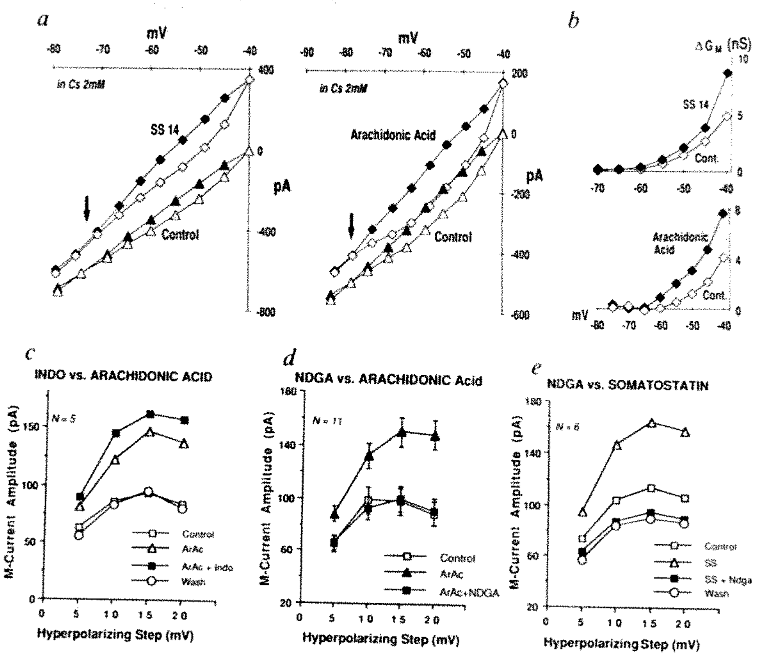
of three neurons bathed with 2 mM Cs^+ (Fig. 2*a*). Neither somatostatin nor arachidonic acid significantly altered reversal potentials for the I_M relaxations (Fig. 2*a*). At holding potentials of −40 mV, both somatostatin and arachidonic acid increased M conductance from about 4–5 (control) to 8–9 nS (Fig. 2*b*). Although there was a trend for the I_M time constant to increase with superfusion of either somatostatin or arachidonic acid (cf ref. 5), this effect did not reach significance when averaged across all responding cells (data not shown).

Superfusion of indomethacin (8–10 μM) to block cyclooxygenase action (an alternative branch of the arachidonic acid pathway, leading to prostanoids) did not significantly antagonize somatostatin-induced (eight cells) or arachidonic acid-induced (five cells; Fig. 2*c*) augmentation of I_M. Indeed the somatostatin (two cells) and arachidonic acid effects (two cells) were sometimes increased by indomethacin (Fig. 2*c*); indomethacin alone had no effect on I_M (three cells). Cyclooxygenase blockade might lead to shunting of arachidonic acid toward the lipoxygenase path[14], thus accounting for the occasional enhancement of somatostatin effects seen with indomethacin. Prolonged indomethacin superfusion times (15–20 min), however, reduced somatostatin-induced increases in I_M to below control levels in four out of eight cells. Such late actions could reflect nonspecific inhibition of PLA$_2$ (ref. 15) as long superfusions with indomethacin never reduced increases in I_M[15] elicited by arachidonic acid. Superfusion of prostaglandins E_2, $F_{2\alpha}$ or I_2 (2–60 μM) did not significantly alter I_M (seven cells; data not shown). Taken together, these findings suggest that a cyclooxygenase product of arachidonic acid is probably not involved in the somatostatin effect on I_M.

Superfusion of nordihydroguaiaretic acid (NDGA, 10 μM) a lipoxygenase inhibitor[16], abolished both the somatostatin-induced (five of six cells; Fig. 2*e*) and the arachidonic acid-induced (11 of 11 cells; Figs 2*d* and 3*a*) I_M augmentation, bringing the baseline I_M back to control levels. NDGA superfused alone had no significant effect on I_M in four additional cells. Furthermore, long superfusion times of somatostatin alone (up to 25 min) did not decrease the somatostatin-induced effect on I_M.

Several lipoxygenase products are candidates for mediation of somatostatin effects. Superfusion of the 5-lipoxygenase product leukotriene C_4 (LTC_4; 5–8 μM), even in the presence of NDGA, mimicked the action of somatostatin and arachidonic acid in augmenting both the M-current and input conductance, and in eliciting an outward baseline current, in four of five cells (Figs 3*a, b*). Leukotriene B_4 (15–30 μM) had no significant effect on these parameters (four cells; Fig. 3*c*). In preliminary studies, both 5,6-methanoleukotriene A4 methylester (10 μM) and

FIG. 2 *a*, Current-voltage curve constructed from the instantaneous (filled symbols) and steady state currents (open symbols) for two different cells before (control: triangles) and during (diamonds) superfusion of either SS14 (left panel) or arachidonic acid (right panel). Both experiments in the presence of 2 mM Cs^+ to block I_Q. Arrows indicate the reversal potential for I_M (−73 mV for the left and −79 mV for the right cell), which are very similar to those previously reported for hippocampal pyramidal neurons[12], and are not signficantly changed by either SS14 or arachidonic acid. Resting potentials were −66 mV (left cell) and −68 mV (right cell). *b*, Curves from the same cells showing the changes in the M conductance (ΔG_M) underlying the current relaxation (closing of M-channels), calculated by dividing the amplitude of relaxation (inverted across the range of voltage steps) by its driving force (holding potential minus reversal potential). Note that the conductance is nearly doubled by SS14 (top panel) or arachidonic acid (lower panel), compared to controls (Cont.). *c*, Average I_M data (±s.e.m.) from five cells tested with indomethacin (Indo; 8–10 μM) plus arachidonic acid (ArAc; 120–150 μM): addition of indomethacin actually increases I_M beyond the augmentation elicited by arachidonic acid alone. *d*, Average data from 11 cells tested with arachidonic acid plus NDGA. NDGA (10 μM) significantly ($P<0.01$) blocks the I_M increasing effects of arachidonic acid (120–150 μM) (see Fig. 3*a* for digitized record of NDGA block of arachidonic acid). *e*, NDGA (10 μM) significantly ($P<0.01$) blocks effects of SS14: average data from six cells. In *c* and *e*, individual standard error bars removed for clarity: s.e.m. averaged from all the data is 19.3 pA for *c* and 20.8 pA for *e*.

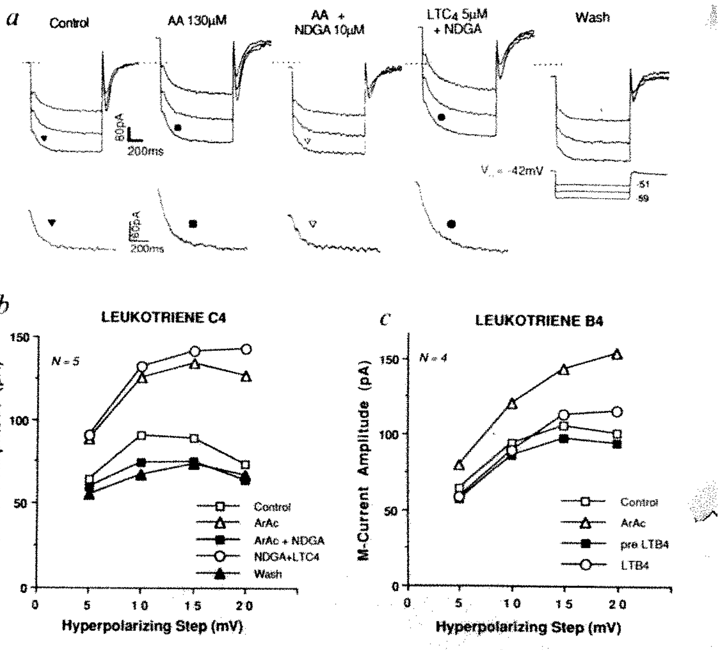


5,6-dehydroarachidonic acid (10 μM), specific 5-lipoxygenase inhibitors[17], completely blocked the M-current augmenting effects of somatostatin (three cells) and arachidonic acid (one cell).

Augmentation of I_M by cyclic AMP was recently reported for smooth muscle[5]. In our present studies of hippocampal neurons, however, superfusion of cyclic nucleotides (8-bromo cyclic GMP; 8-bromo cyclic AMP; dibutyryl cyclic AMP) had no consistent effect on the amplitude of I_M at concentrations up to 2.5 mM, although occasional cells showed a small increase.

Thus, our combined data suggest that some lipoxygenase metabolite of arachidonic acid may be a second messenger or mediator for the I_M-augmenting action of somatostatins in hippocampal pyramidal neurons, and therefore also for the somatostatin-induced hyperpolarizations[4]. The actual metabolite or metabolites involved in hippocampal somatostatin effects is not known, although our data with LTC_4 suggests that a leukotriene is the best candidate. LTC_4 can be metabolized further to LTD_4 and LTE_4, which might also be involved. Our preliminary findings that specific 5-lipoxygenase inhibitors antagonize both somatostatin and arachidonic acid-induced I_M effects are consistent with leukotriene mediation of somatostatin effects. As far as we know, this is the first electrophysiological evidence for such eicosanoid mediation of a transmitter effect in a vertebrate central neuron. Prostaglandins have been implicated in mediation of the biochemical effects of vasoactive intestinal peptide in brain slices[18], and lipoxygenase products may regulate this peptide's release[19]. Piomelli *et al.* have shown that 12-lipoxygenase metabolites may mediate FMRFamide effects on a K^+ current[8] and hyperpolarizations elicited by histamine[20] in *Aplysia* neurons. In addition, leukotrienes have been shown to alter spontaneous extracellular firing rate in rat cerebellar Purkinje cells *in vivo*[21].

FIG. 3 LTC_4, but not LTB_4, mimics arachidonic acid (ArAc) and somatostatin effects on I_M. *a*, Top row: digitized current records from a CA1 pyramidal neuron. Arachidonic acid superfusion (130 μM) augments I_M, increases conductance and causes an outward baseline current. Superfusion of NDGA 10 μM (with arachidonic acid) completely reverses these effects. Subsequent superfusion of LTC_4 (5 μM) in the continued presence of NDGA again augments I_M. Washout brings all current measures back to near-normal levels. Lower row: single I_M relaxations isolated from the upper current traces (with matched symbols) and magnified for clarity. *b*, Average of I_M amplitudes for five cells tested with LTC_4, using holding potentials of about −45 mV, and the same drug protocol as in *a*. Average I_M is significantly increased ($P<0.05$) by LTC_4. *c*, Average I_M amplitude from four other cells tested with LTB_4 (15–30 μM), which has no significant effect on I_M ($P>0.1$). 'Pre-LTB_4' is the 12–20 min period between arachidonic acid and LTB_4 superfusions for washout of ArAc (two cells) or arachidonic acid blockade with NDGA 10 μM (two cells). In *b* and *c* standard error bars removed for clarity; average s.e.m. from all the data is 19.6 pA for *b* and 8.5 pA for *c*.



To our knowledge there are no reports showing biochemical evidence of somatostatin-induced increases in PLA_2 or lipoxygenase activity. It is possible that a GTP-binding protein may be involved in activation of PLA_2 (ref. 22). Leukotrienes are synthesized in several regions of the brain, including the hippocampus[23]. Specific LTC_4 binding sites are also found in several brain regions and are among the most numerous in the hippocampus; binding sites for LTB_4 and LTE_4 are weak and those for LTD_4 undetectable[24]. The exact subcellar location of receptors for the lipids however, is still unknown: given the great membrane permeability of the lipids, they could act on the M-channel by either an intracellular or extracellular route, as suggested by others[8,25,26]. Thus, presynaptic or feedback effects cannot be excluded. Although the exact physiological role and site of action of the relevant lipid(s) cannot be stated at this time, it seems likely that, as in some invertebrate neurons[8,20], a single ion channel type in mammalian central neurons can be regulated in opposing directions by two different ligands (somatostatin and acetylcholine) acting through different second messenger systems. Such bidirectional regulating mechanisms should provide sensitive biasing of ion channel activity, and therefore neuronal excitability. □

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Dual-component NMDA receptor currents at a single central synapse

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PRESENT thinking about the way that the NMDA (*N*-methyl-D-aspartate) class of glutamate receptor operates at central synapses relies mainly on information obtained from single-channel and whole-cell recordings from cultured neurons stimulated by exogenous NMDA receptor agonists[1,2]. The mechanisms that operate in the postsynaptic membrane of a normal neuron following release of the natural transmitter are far less clear. An important problem is that most normal neurons receive many excitatory synapses (10^3–10^5 per cell) and these synapses are located on slender dendritic elements far away from the somatic recording site, making the study of discrete synaptic events difficult. Typically, when populations of synapses are activated, NMDA receptor-mediated synaptic potentials appear as slowly rising, long-lasting waves superimposed on faster, non-NMDA-receptor potentials[2–8]. Although believed to be critical for NMDA receptor function[9,10], this slow time-course would not be predicted from single-channel kinetics and its origin remains puzzling. We have now analysed the events occurring at the level of a single excitatory synapse using a simple, small, neuron—the cerebellar granule cell— which has an unusually simple glutamatergic[8] input. By applying high-resolution whole-cell recording techniques to these cells *in situ*[11], we were able to study the nature of elementary NMDA receptor-mediated synaptic currents. Contrary to expectations, the prominent currents are fast but are followed by slow ones. Both types of current are strongly voltage-dependent but differ subtly in this respect. Furthermore, the currents are absent unless glycine is provided.

Cerebellar granule cells are only about 6 μm in diameter and receive a simple monosynaptic excitatory input from mossy fibres, which originate mainly in nuclei in the brain stem and spinal cord. The total number of excitatory synaptic boutons supplied to each cell amounts to only 3 or 4, one on each short dendrite[12,13]. Granule cells near the surface of parasagittally cut rat cerebellar slices were selected for study. One or two of the dendrites of these more superficial cells would have been cut off, leaving one or two synapses buried within the slice (Fig. 1*a*). The patch electrodes contained Nystatin to obtain 'perforated' whole-cell recordings, which minimize loss of cytoplasmic components[14], and CsCl as the main electrolyte. All bathing solutions contained tetrodotoxin and bicuculline to block action potentials and inhibitory currents. The membrane capacitance was 3.3 ± 0.37 pF (mean ±s.d. of 87 cells in 11 slices) which, assuming a specific capacitance of 1 $\mu F\ cm^{-2}$, gives an equivalent mean spherical cell diameter of just 10 μm.

The most obvious currents were fast and of a shape suggesting them to be synaptic currents produced by spontaneous release of quanta of transmitter from presynaptic terminals[15,16]. Consistent with this interpretation, their frequency was increased by perfusion of K^+ to depolarize the nerve terminals (Fig. 1*c*) and their amplitude distribution was bell-shaped (data not shown). The currents were identified as NMDA receptor-mediated on the basis of the following observations.

First, the currents were absent unless glycine was supplied (Fig. 1*b*). Low concentrations of glycine augment responses to NMDA by binding to a high-affinity site on the NMDA receptor[17]. In cultured neurons, fast perfusion techniques are needed to prevent activation of this site by glycine continuously released from nearby cells. In our initial tests, the incubation bath (1.5 ml capacity containing 3–5 slices) was left static for 0.5–3 h before recording. Nevertheless, all 17 cells studied (4 slices) were initially silent. But 14 of them displayed spontaneous currents on local addition of glycine. With 10 μM glycine in a continuously perfused medium (subsequently adopted), 70% of recorded cells displayed spontaneous currents.

Second, the currents were reversibly abolished by the NMDA antagonist APV (D-2-amino-5-phosphonovalerate; Fig. 1*d*), but

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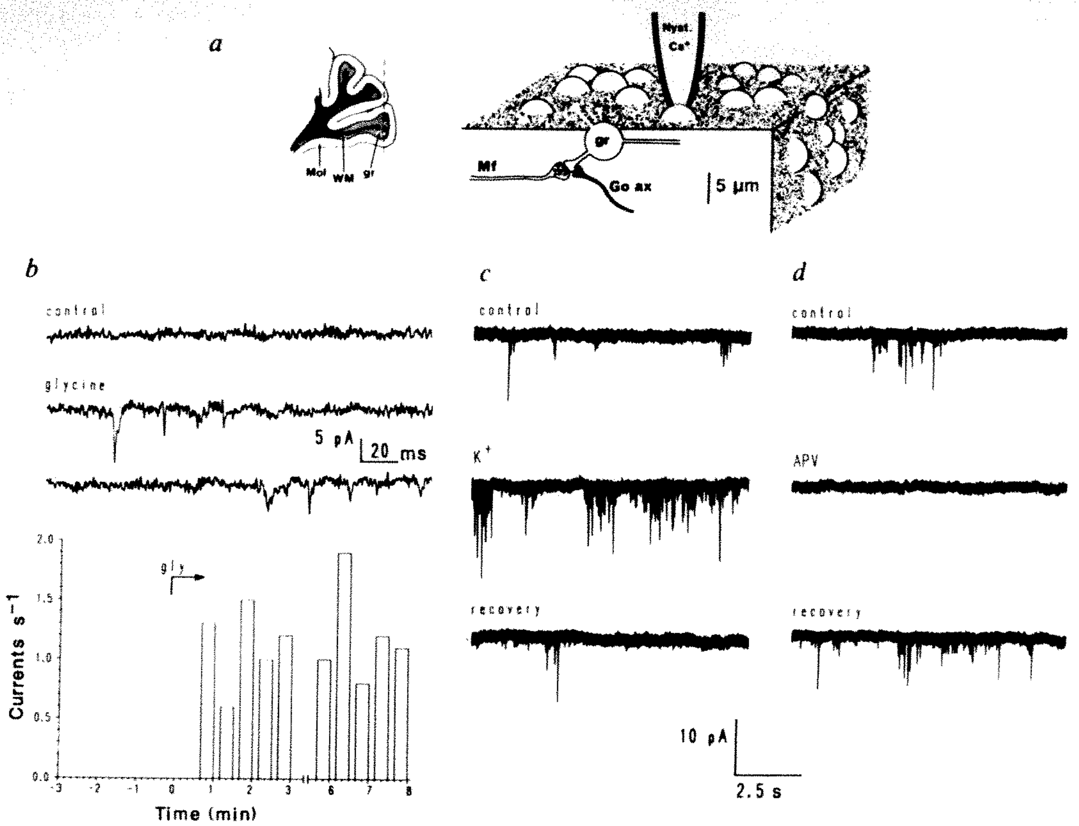

FIG. 1 Spontaneous synaptic activity is revealed by addition of glycine (*b*) and is enhanced by K⁺ (*c*) and abolished by APV (*d*). Whole-cell recordings were made from granule cells in thin slices of rat cerebellum (shown schematically in *a*) but no activity could be observed, in this case recording over 10 min with the slice bathed for 2 h in nominally Mg^{2+}-free medium, until glycine was delivered from a pipette (containing the amino acid at a concentration of 10 μM) positioned 50–100 μm from the recorded cell. In a different experiment, K⁺ and APV were also perfused locally (30 mM and 100 μM in the pipette, respectively) with 10 μM glycine and 1.2 mM Mg^{2+} in the bath. The effect of APV was observed all five cells tested and, with K⁺, quantitation of current frequency (currents · s^{-1}) showed a significant increase (2- to 16-fold; three cells) within the first 30 s of application. The holding potential was −40 mV in all cases and the currents were filtered at 2 kHz. The currents were unaffected when 10 μM strychnine, an antagonist acting at inhibitory glycine receptors, was perfused together with glycine (six cells, two slices; see also Fig. 3 legend). *a*, Left: Mol, molecular layer; WM, white matter; gr, granule cell layer. *a*, Right: Mf, mossy fibre; gr, granule cell; Go ax, Golgi cell axon.

METHODS. Thin (70–120 μm) parasagittal slices of cerebellar vermis from young (12–15-day-old) rats were prepared using a Vibroslice[11]. A microknife cut was made across the upper part of the granule cell layer (see Fig. 1*a*) to improve visibility (Olympus CK2 inverted microscope equipped with phase contrast optics) and sever the long granule cell axons. The slices were transferred to a polylysine-coated Petri dish containing 1.5 ml of a solution maintained at 30 °C and containing NaCl (130 mM), KCl (3 mM), Na_2HPO_4 (1.2 mM), $CaCl_2$ (2 mM), $MgSO_4$ (1.2 mM), glucose (11 mM), Tris-HCl (15 mM) (pH 7.4), tetrodotoxin (1 μM) and bicuculline (10 μM). In some experiments, $MgSO_4$ was omitted. The slices were immobilized by nylon threads fixed to a silver ring. A stream of prewarmed solution was gently superfused from a local pipette over a selected folium for 10–30 min. Except in the earlier experiments (see text) the bathing medium was continuously exchanged (1 ml min^{-1}). The recording microelectrodes (10–12 mΩ) were filled with a solution containing Nystatin (100 μg ml^{-1}) and CsCl (140 mM), NaCl (4 mM), $CaCl_2$ (0.5 mM), EGTA (5 mM), HEPES buffer (5 mM), pH 7.2 (CsOH). The Nystatin method was adopted both for theoretical reasons[14] and because the success rate in gaining access to the cell interior was considerably higher than with conventional whole-cell recording. Whole-cell currents were derived using a List EPC7 amplifier. The access resistance averaged 70 MΩ and the steady-state input resistance was typically 10 GΩ.

were unaffected by CNQX (6-cyano-2,3-dihydroxy-7-nitroquinoxaline) which is a relatively selective non-NMDA antagonist in the presence of 10 μM glycine[18] (see Fig. 3*b*). Third, with a physiological Mg^{2+} concentration (1.2 mM) in the bathing solution, current-voltage relationships (Fig. 3*a*) were linear ($R = 0.99$) from positive holdings to −20 mV, reversed at −6.5 mV (uncorrected value; see Fig. 3 legend) and, as is characteristic of NMDA currents[1,2], displayed a negative slope conductance between −30 and −70 mV. In the absence of added Mg^{2+}, the curve was linear from positive potentials down to −40 mV and the currents thereafter were larger than with Mg^{2+} added (Fig. 3*a*), consistent with a block of the NMDA channels by this ion and the presence of residual Mg^{2+} in the nominally Mg^{2+}-free extracellular solution. Over the linear range, the slope was 255 pS (1.2 mM Mg^{2+}), 254 pS (no added Mg^{2+}) and 246 pS (1.2 mM Mg^{2+}, 10 μM CNQX). Assuming a single channel conductance of 50 pS[1,2], the fast currents are composed, on average, of the synchronous opening of five NMDA channels.

Kinetic analysis of these currents resulted in mean rise times (measured between 20–80% of the peak; means ±s.d.) of 0.57 ± 0.65 ms at −60 mV and 0.77 ± 0.55 ms at +50 mV ($n = 10$). The corresponding times to peak were 0.77 ± 0.65 ms and 1.13 ± 0.55 ms. These rapid kinetics are reminiscent of those of spontaneous endplate currents recorded at the neuromuscular junction[15]. The decay of the individual currents, however, was complex, as expected from the random closure of so few NMDA channels. Digital averaging (Fig. 2*b*) suggested a decay time constant (τ_d) at +50 mV of 7.1 ± 0.9 ms (mean from best exponential fits of the averaged current decay in three cells ±s.d.). This value is in good agreement with the reported mean lifetime of unblocked NMDA channels[19,20]. The decay became faster at negative potentials (Fig. 2*b*); at −50 mV, τ_d was 3.1 ± 1.1 ms ($P < 0.02$). This could reflect a reduced channel-burst duration at negative membrane potentials.

Beyond the main peak, the kinetics of single currents were not easily resolved. This was because the rapid events were often followed by a noisy tail, highly variable in duration, but frequently lasting for tens of milliseconds (Fig. 2*a*). In averaged records, these tails generated a 'hump', peaking 20–25 ms after the start of the fast component (Fig. 2*b*). The current-voltage plot of the hump over the range +50 to −70 mV was like that of the fast current (Fig. 3*c*), but between −70 and −80, the fast current increased whereas the hump decreased. The ratios of the currents at these two potentials were 1.90 ± 0.31 for the fast component and 0.84 ± 0.29 for the hump (means ±s.d.; data from Fig. 3*a* and *c*; $P < 0.002$).

It can be concluded that quanta of excitatory transmitter are released spontaneously at this central synapse and that they elicit postsynaptic currents exclusively through NMDA receptors. It is likely that other synapses are similar in this respect as 'tonic' NMDA receptor activation has been detected as a background current in hippocampal neurons in the absence of synaptic stimulation[21]. Such spontaneous activity could have an important influence on neuronal excitability[21]. Non-NMDA receptors are present on granule cells and can be activated following electrical stimulation of mossy fibres[8], but they may need a higher concentration of transmitter to become effective[19].

The primary (fast) synaptic current elicited through NMDA receptors has largely predictable kinetics, assuming quanta of transmitter bind to receptors located close to the release site and so cause the synchronous opening of about five NMDA channels. The subsequent slow current, however, is likely to be the one responsible for the slow synaptic NMDA potentials previously detected in this and several other pathways following electrical stimulation of afferent fibres[2-8]. We can now exclude several hypotheses that have attempted to explain the slow time-course of this component by invoking network-related factors, such as polysynaptic pathways[2]. Instead, an attractive possibility is that after the channels mediating the fast component have closed, the transmitter dissociates and then rebinds to receptors asynchronously[22]. Given the delay to the peak of the slow wave, the operative receptors could be located some distance away, perhaps extrasynaptically. One prediction of this hypothesis is that the time to the peak of the slow wave should shorten as the decay rate of the fast currents increases, as indeed the averaged records (Fig. 2*b*) suggest.

Finally, the precise action of glycine and whether the ambient levels of glycine are limiting for NMDA receptor function or not, are both controversial issues[23]. Our results strongly suggest that glycine is an obligatory co-agonist at the synaptic NMDA receptor[24], as no currents could be detected in the absence of added glycine. This, in turn, implies that the ambient levels in the synaptic cleft are very low, probably ≤30 nM (from glycine dose-response curves[25]). Although it cannot be excluded that diffusion of glycine out of the slice is responsible, our findings with preparations bathed for long periods in unstirred solutions would not favour this possibility. Moreover, the availability of glycine is limiting for NMDA receptor function in the cerebellum *in vivo*[26]. Thus, it is more likely that there is a mechanism maintaining glycine at a low concentration in this synapse. The axon terminals of Golgi cells are equipped with a high-affinity glycine carrier[27] and are in an anatomically appropriate position to undertake this function. Being rich in glycine[28] the same terminals could supply the amino acid to the synapse under appropriate conditions and so regulate granule cell NMDA currents. □

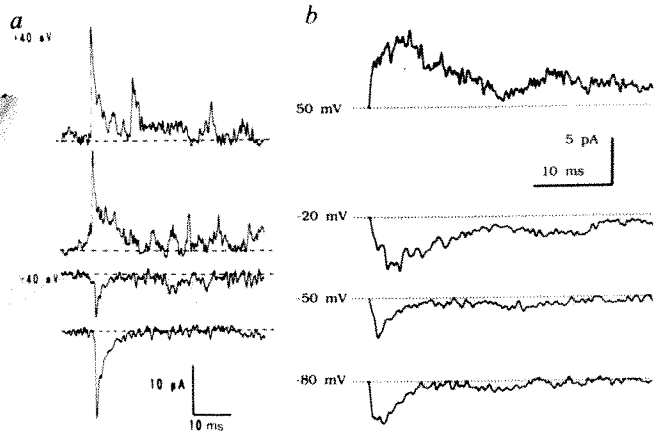

FIG. 2 Properties of spontaneous currents. *a*, Individual currents recorded at +40 and −40 mV (1.5-kHz low-pass filter). Note the 'noise' appearing after the decay of the initial fast current in the upper three traces. When averaged (*b*) this noise generates a hump. Note also in *b* that the decay of the fast component and the time to peak of the hump seem to be shortened by hyperpolarization and that the voltage dependence of the peak of the fast component differs from that of the hump (compare −50 and −80 mV). The perfusing solution contained 10 μM glycine, 1.2 mM Mg^{2+} and (in *b*), 10μM CNQX as well. For each record in *b* 8–15 single currents were digitized at 20 kHz and averaged off-line by synchronizing and zeroing them at the start of the fast current. All events having an amplitude equal to, or greater than, twice the background noise were included in the averages. The data are from the same cell except for that at +50 mV.

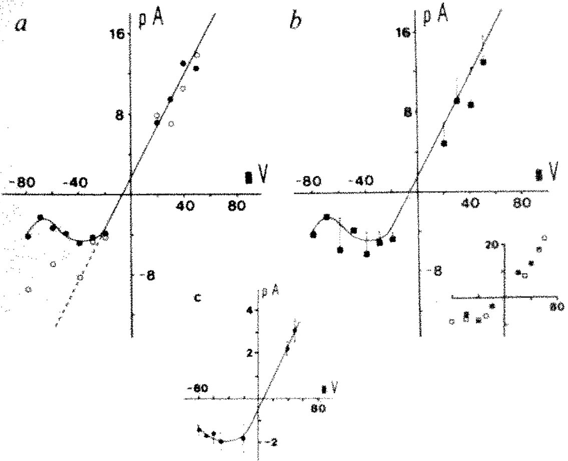

FIG. 3 Mean current–voltage (*I*–*V*) relationships for the fast current (*a*, *b*) and the hump (*c*). In *a* the bathing solution contained either 1.2 mM Mg^{2+} (filled circles) or no added Mg^{2+} (open circles). Standard deviations are omitted for clarity but averaged 24% of the means. The data in *b* (±s.d.) were from cells incubated with 1.2 mM Mg^{2+} and 10 μM CNQX. Each point (*a* and *b*) represents the mean peak amplitude of individual currents in five cells (two different slices). All currents of amplitude greater than or equal to twice background noise were included in the analysis; the mean current at −70 mV in Mg^{2+}-containing solution may thus be an overestimate (noise level, 1–1.5 pA). The current amplitudes were not significantly affected by strychnine; for example, at +40 mV, with no added Mg^{2+} and 10 μM strychnine in the perfusing solution, the mean current in three cells (±s.d.) was 8.4 ± 2.2 pA. The inset (*b*) shows the *I*–*V* curve for a single cell in the presence (solid symbols) and absence (open symbols) of 10 μM CNQX. The *I*–*V* curve for the hump (*c*) was obtained from measurements of its peak amplitude in averaged currents (Fig. 2*b*) from four different cells (means ±s.d.). The solid line (*a* and *b*) fits data points in 1.2 mM Mg^{2+}, zero CNQX (by linear regression at potentials above −20 mV, the remainder by eye). The apparent reversal potential for these currents is −6.5 mV. In the perforated patch whole-cell recording configuration, a Donnan equilibrium will develop between the pipette and the cytoplasm, which should make the pipette 8.9 mV positive[14] and thus shift *I*–*V* relationships to the left by this value. As the reversal potential of the NMDA current (considering only the Nystatin-permeant ions in the pipette and in the bath) can be calculated to be +4.3 mV (ref. 29), the synaptic currents should theoretically reverse at −4.6 mV, which is in reasonable agreement with the experimental value.

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Linkage of a nasopharyngeal carcinoma susceptibility locus to the HLA region

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THE frequency of nasopharyngeal carcinoma is nearly 100-fold higher in southern Chinese than in most European populations[1]. Earlier studies have suggested that an increased risk of nasopharyngeal carcinoma is associated with specific haplotypes in the HLA region: relative risks slightly over twofold were found for haplotypes A2, Bw46 and the antigen B17 (refs 2–4). We now report a linkage study based on affected sib pairs which suggests that a gene closely linked to the HLA locus confers a greatly increased risk of nasopharyngeal carcinoma. The maximum likelihood estimate is of a relative risk of approximately 21. The relationship between this suspected disease susceptibility gene (or genes) and known viral and environmental aetiological factors remains to be elucidated.

Our approach was to identify sibships that had more than one individual affected with nasopharyngeal carcinoma (NPC), and for which sufficient individuals could be typed to permit unambiguous assignment of haplotypes[5]. The study began in Singapore and Hong Kong in 1976 and extended in 1983 to Nanning in the Guangxi Autonomous region of the People's Republic of China, where more sib pairs were accessible (see Table 1 legend). In each area, the members of each sibship, their parents and children were visited and blood samples taken. Thirty-four sibships with more than one case of NPC were identified, 31 with two cases and the remainder with three cases (Table 1). Of the sib-pair families, four had to be excluded, one because the pair were twins of unknown zygosity (but HLA identical) and three because an excess of haplotypes was seen in the family and parentage was ambiguous.

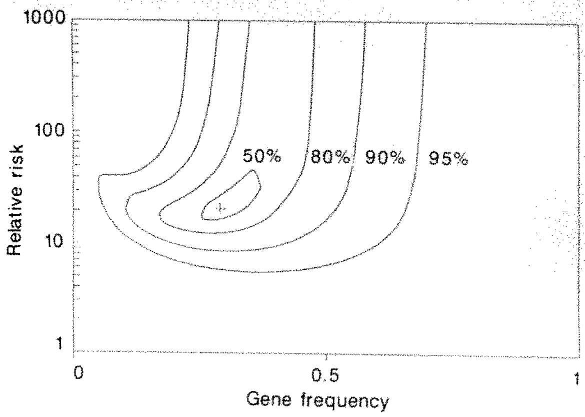

FIG. 1 For the model fitting, inference was based on the log-likelihood difference conditional on the disease phenotypes, or lod score. The use of this conditional likelihood allows inferences to be made free from ascertainment bias[6,8]. Likelihood calculations were performed using the program LINKAGE[7] for single-gene dominant and recessive models, varying the gene frequency relative risk associated with the disease susceptibility gene, and the recombination fraction θ between the susceptibility gene and the HLA region. In particular, the lower 95% confidence limit for the relative risk associated with the susceptibility gene, r_0, is given by:

$$2\left[\max_{r,p,\theta} l(r,f,\theta)-\max_{p,\theta} l(r_0,f,\theta)\right]=3.84$$

where $l(r,f,\theta)$ denotes the log-likelihood function and 3.84 is the upper 95% point for a χ^2 distribution on 1 degree of freedom. The contours shown define confidence regions for r and f, at the 95%, 90%, 80% and 50% level with $\theta=0$. The absolute maximum of the likelihood occurs when $\theta=0$, with values of $f=0.29$, $r=20.9$, and is marked by a cross (+).

HLA typing on the samples from Hong Kong and Singapore was performed in Singapore and restricted to locus A and B antigens, using a panel of over 200 antisera of Chinese, Malay, Japanese, Filipino and Caucasian origin. HLA typing in Nanning used a panel of 143 allelic typing antisera (from Saint Louis Hospital, Paris) corresponding to HLA A, B, C and in most cases DR loci. Of the 38 NPC cases tested, the most common DR antigens were DR2 (18/38) and DR4 (16/38). No

TABLE 1 Sib-pair analysis

	Number of shared haplotypes 2				1				0			
		Expected under				Expected under				Expected under		
	Observed	No linkage	Dominant	Recessive	Observed	No linkage	Dominant	Recessive	Observed	No linkage	Dominant	Recessive
Sib pairs*	16	6.75	10.83	13.96	8	13.50	13.18	10.40	3	6.75	2.99	2.64
Triples†	2	2.25	3.30	4.01	4	4.50	4.39	3.91	3	2.25	1.31	1.08
Total	18	9.00	14.13	17.97	12	18.00	17.57	14.31	6	9.00	4.30	3.72

Number of sib pairs sharing two, one or zero HLA haplotypes identical by descent, together with the expected numbers under the null hypothesis of no linkage, and under the best fitting dominant and recessive models. For the sib trios, all possible sib pairs are considered.

Goodness of fit[11] of the hypothesis of no linkage, χ^2 (2 d.f.) for sib pairs only $=17.0 P<0.001$; for all pairs (both sib pairs and triples) $=12.0 P<0.005$. Goodness-of-fit tests of the recessive and dominant models are each given by a 1 d.f. likelihood ratio test against a three-parameter model, with relative risk r_1 for the DS heterozygote, and r_2 for the DS homozygote. χ_1^2 for recessive model $=0.32$; χ_1^2 for dominant model $=2.95$ ($P=0.08$).

* 18 from China, 3 from Hong Kong, 5 from Singapore and 1 from Malaysia.

† 2 from China and 1 from Hong Kong.

other DR antigen was present on more than seven individual cases.

The haplotype similarity of affected sibs and triplets is shown in Table 1, together with the expected numbers if no linkage were present. The total data show a clear divergence between observed and expected ($P < 0.005$). Among the sib pairs, comparing observed with expected gives a χ^2 value of 17.0 (2 d.f.). This highly significant divergence demonstrates the existence of NPC disease susceptibility gene(s) linked to the HLA region.

For linkage analysis, single-gene models were explored[6–8], and as models assuming dominant inheritance fitted poorly (see Table 1), the subsequent analyses were based on recessive models. Figure 1 shows the likelihood of the observed results (lod score) for varying values of the relative risk (r) and the gene frequency (f) of the putative disease susceptibility gene when the recombination fraction (θ) is zero. The maximum lod score under the recessive model is +2.39, and under the dominant model +1.68, with P values of 0.004, and 0.024 respectively.

The maximum likelihood estimate occurs when $\theta = 0$, $r = 20.9$ and $f = 0.29$. Infinite values of r are only slightly less likely for a range of values of f; the minimum value of r consistent with the data is 5.1 at the 95% level, and 9.8 at the 90% level. With values of θ greater than zero, all the likelihood contours are further removed from the axis given by $r = 1$.

These results show the existence of an NPC disease susceptibility gene(s) conferring an increased risk for NPC some tenfold larger than that associated with Bw46 or B17, with the upper 90% confidence bound being infinite. The results thus provide strong support for the original hypothesis[5] that much of the high risk for NPC among the southern Chinese is due to HLA-associated genes. The lifetime risk of NPC among Cantonese males in Singapore[1] is 1.6%, indicating that the frequency of a recessive disease susceptibility gene should be at least 0.13.

The mechanism by which an HLA-linked disease susceptibility gene might act is unknown. Two proposed environmental aetiological agents for NPC are the Epstein–Barr virus (EBV) and some constituents of preserved foods, including salted fish[8–10]. Insight into the mechanism of action of NPC disease susceptibility gene(s) should help to clarify the respective role of EBV, environmental cofactors and genetic susceptibility in the development of NPC. □

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Residues of the variable region of the T-cell-receptor β-chain that interact with *S. aureus* toxin superantigens

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THE αβ T-cell antigen receptor (TCR) recognizes antigenic peptides in the context of self major histocompatibility complex (MHC) molecules[1–4]. The specificity of recognition of MHC plus antigen is generally determined by a combination of the variable elements of α- and β-chains of the TCR[5–8]. Several types of antigen, however, have been identified that, when bound to MHC molecules, stimulate T cells bearing particular variable-region β-chain (Vβ) elements irrespective of the other variable components of the TCR[9–21]. These have been termed 'superantigens', and here we are concerned with one type of superantigen, the toxins produced by *Staphylococcus aureus*. T cells have been found that bear closely related members of the same Vβ family but respond differently to *S. aureus* toxins[15,19,22]; in particular, cells bearing the human Vβ13.2 element respond to toxin SEC2, whereas cells bearing human Vβ13.1 do not. We have now defined the residues of the Vβ element responsible for this difference, and find that they reside in a region thought to lie on the side of the TCR molecule, away from the conventional antigen/MHC-binding site. The evolutionary conservation of this site may be due to its having an important role in some function of the TCR other than the binding of conventional antigen plus MHC.

For these structure-function studies, we designed a transfection system in which the products of human *Vβ* genes were introduced into an otherwise murine TCR expressed on the surface of a mouse T-cell hybridoma (see legend to Fig. 1). The specificity of this chimaeric receptor could then be determined by the ability of the transfectants to produce interleukin-2 (IL-2) when stimulated with various toxins presented by cells bearing class II MHC molecules. Initially, transfectants were produced with receptors bearing either human (h) Vβ13.1 or Vβ13.2. Representative clones with good TCR surface expression were tested for reactivity to toxins presented either by A20, an H-2^d class II$^+$ murine B-cell lymphoma (Table 1), or by murine splenic class II$^+$ non-T cells of various H-2 haplotypes (Table 2). Immobilized anti-Cβ antibody (that is, an antibody to the TCR β-chain constant residues) was used as a positive control to measure maximal TCR-mediated stimulation. The Vβ13.1-bearing transfectant failed to respond to any of the toxins presented by any of the murine presenting cells, although it responded well to immobilized anti-Cβ antibody. The Vβ13.2 transfectant responded to the toxin SEC2 and less well to SEC3 regardless of the presenting cells. These differences in reactivity were seen over a range of toxin concentrations (data not shown).

These results establish several points. First, they show that human-murine chimaeric β-chains assembled properly with murine α-chains and murine CD3 to yield functional receptors. Second, the specificities of the human Vβ chimaeric receptors are consistent with those seen in normal human T-cells[19]. Finally, for an individual TCR, interaction with the allelic residues in the MHC portion of the toxin-MHC ligand can influence the degree of response[15,23]. The finding that T cells bearing Vβ13.1 and Vβ13.2 respond differently to the toxins SEC2 and SEC3 regardless of the allelic form of murine class II used for presentation, suggests that the residues that differ between these two Vβ regions are involved in toxin rather than MHC interaction.

The amino-acid differences between Vβ13.1 and Vβ13.2 are shown in Fig. 2 superimposed on a schematic drawing of a portion of the structure of Vβ, predicted by Chothia *et al.* on the basis of the conservation of critical residues between Vβ and the variable region of the immunoglobulin heavy chain, V$_H$[24]. There are two main stretches of amino acids at which the two Vβ regions differ. One is predicted to lie in the second complementarity determining region, CDR2—part of the

TABLE 1 Response of transfected T-cell hybridomas to *S. aureus* toxins

Vβ-transfected T-cell hybridoma	αβ TCR density*	Units ml^{-1} IL-2 produced in response to A20-11.1 and *S. aureus* toxins										
		None	SEA	SEB	SEC1	SEC2	SEC3	SED	SEE	ExT	TSST-1	Anti-TCR
hVβ13.1-1	34	—†	—	—	—	—	—	—	—	—	—	843
hVβ13.2-1	19	—	—	—	—	86	44	—	—	—	—	256
hVβ13.1D2-1	30	—	—	—	—	678	200	—	—	—	—	725

Three T-cell hybridomas bearing different hVβ regions were tested for their ability to respond with IL-2 production to either nine different *S. aureus* toxins presented by a murine H-2^d B-cell lymphoma (A20-11.1) or an immobilized anti-TCR antibody (H57-597), as previously described[25]. The limit of detection was 10 U IL-2 ml^{-1}. T-cell hybridomas hVβ13.1-1, hVβ13.2-1 and hVβ13.1D2-1 express hVβ13.1, hVβ13.2 and hVβ13.1D2 respectively. The concentrations of toxins used were: 10 μg ml^{-1} for SEB, SEC1, SEC2, SEC3 and SED; 1 μg ml^{-1} for SEA, ExT and TSST-1.

* αβ TCR receptor density is presented as mean fluorescence of T cells stained with biotinylated H57-597 plus phycoerythrin-streptavidin.

† <10 U IL-2 ml^{-1} indicated by —.

receptor thought to be responsible for binding ligands of conventional antigenic peptides complexed to MHC. The other stretch of differences lies in a region of the β-chain that, on the basis of the immunoglobulin model, would form part of the side of the β-chain, exposed to the aqueous phase and well away from the peptide antigen/MHC-binding site or the interaction points between Vβ and Vα. We decided to concentrate on this part of Vβ, partly because amino-acid residues in it contribute to the interaction between Vβ and the mouse endogenous superantigen, Mls-1^a (ref. 20). Therefore, we prepared a third transfectant, hVβ13.1D2, in which the unique residues of Vβ13.2 from positions 67–77 were introduced into Vβ13.1.

The transfectant bearing this mosaic Vβ showed the same pattern of reactivity to the panel of toxins and presenting cells as that seen with the Vβ13.2 transfectant, that is, high reactivity to both SEC2 and SEC3, but little or none to the other toxins, regardless of the H-2 type of the presenting cell (Tables 1 and 2). The magnitudes of the responses of the transfectant bearing the mosaic Vβ were in fact higher than those seen with Vβ13.2 even when anti-Cβ antibody was used as the stimulant. This was probably due to a higher density of receptors on the mosaic Vβ transfectant than on the other cell lines.

Therefore, the introduction of eight amino-acid residues at positions 67–77 of human Vβ13.2 into human Vβ13.1 is sufficient to confer the toxin reactivity of hVβ13.2 on the mosaic Vβ produced, suggesting that the site for toxin-binding on Vβ lies in this region of the molecule, not in the face containing the predicted complementarity-determining regions thought to control conventional peptide antigen recognition. In addition, the fact that this same region of Vβ has been implicated in the binding of an endogenous mouse superantigen, Mls-1^a, to the TCR (ref. 20) suggests that the bacterial toxin and endogenous murine superantigens bind to the same or related sites on Vβ.

None of the structures of the endogenous superantigens are known; the discovery of overlapping binding sites on Vβ may indicate that the endogenous superantigens are indeed proteins, with structures related to those of the toxins. The evolutionary conservation of this superantigen binding site on Vβ is puzzling, as it both offers a target for the bacterial toxins and results in large scale deletion of T cells bearing particular Vβ elements in individuals carrying an endogenous superantigen, although apparently not contributing to recognition of conventional antigen–MHC ligands[9-20]. This site may play an indispensable part in some other TCR function, such as the positive selection of

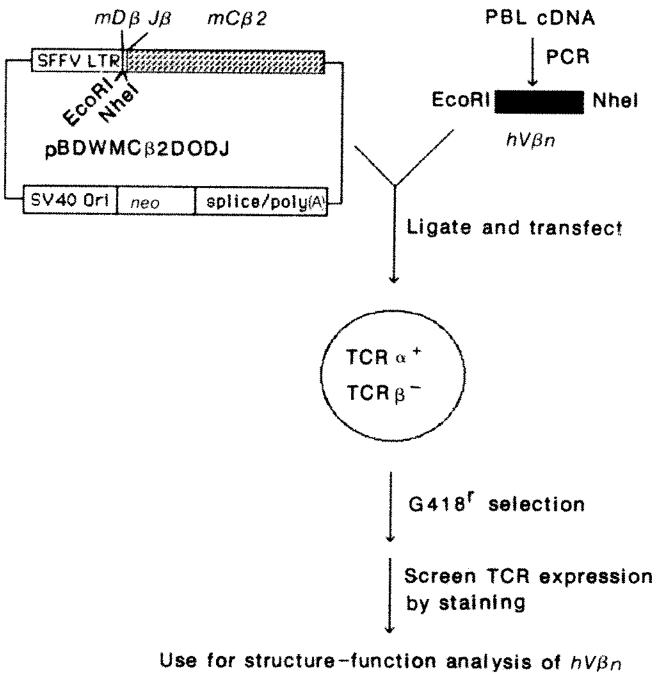

FIG. 1 Scheme for expression of hVβ elements on murine T-hybridoma cells. The studies used an expression vector/host-cell system wherein specific human *Vβ*-containing constructs were expressed on the surface of a murine T-hybridoma cell line. In brief, an expression vector was constructed that carried the mouse *Cβ2* gene and mouse *DβJβ* elements (pBDWMCβ2DODJ). A human *Vβ* region sequence was prepared by amplification of cDNA prepared from human peripheral blood lymphocytes (PBL) stimulated with anti-CD3 antibody[19]. The amplified human *Vβ* region was joined in-frame to the expression vector, pBDWMCβ2DODJ. This new chimaeric TCR β-chain (human V-region, mouse DJ region and *C* chain) was transfected into the recipient mouse T-cell hybridoma, DS23-27.4, which expresses all the functional components of the TCR complex except the TCR β-chain (D.D. and E. Palmer, manuscript in preparation). The transfectants were selected by G418 resistance. The expression of human-mouse chimaeric TCR β-chain was screened by staining with either anti-CD3 (145-2C11) or anti-αβ TCR (H57-597) antibody[25,26].

METHODS. Total RNA and cDNA were prepared as described previously[19]. Primers (5′ and 3′) for the polymerase chain reaction (PCR)[27] carried *Eco*RI and *Nhe*I sites, respectively. Amplified *hVβn* fragments were subcloned into pTZ18R vector, sequenced and ligated into expression vector pBDWMCβ2DODJ. The plasmid pBDWMCβ2DODJ, derived from pSFFVSVneo (gift of Dr S. Fine), contained *DβJβCβ2* cDNA sequences cloned from the mouse hybridoma DO11.10 (ref. 28). The expression vector carrying the appropriate *hVβn* genes was transfected into recipient murine T cells (DS23-27.4) by electroporation[29]. The recipient line, DS23-27.4, was derived from an antigen-specific mouse T-cell hybridoma (DS23.27) by irradiation. The DS23-27.4 had lost its rearranged TCR β-chain genes, as confirmed by Southern blot analysis. The transfected cells were selected with 700 μg ml^{-1} G418. The *hVβ13.1* sequence was aplified with the primers 5′-GGGAATTCAAGATGGCCATCGGCCTCCTGTGCTGTGC-3′ and 5′-TAACTGCTAGCACAGAAGTACACAGATGTC-3′. The *hVβ13.2* sequence was amplified with 5′-GGGAATTCAAGATGGCCCTCGGGCTCCTGTGCTGTGG-3′ and 5′-GCTGCTAGCACAGAAGTACACAGATGTT-3′ oligonucleotides. The sequences of *hVβ13.1* and *hVβ13.2* obtained in this study are consistent with those described previously[30,31]. The *hVβ13.1D2* sequence was derived from *hVβ13.1* and *hVβ13.2* by joining 5′ of *hVβ13.1* sequences to 3′ of *hVβ13.2* at amino-acid position 66 by PCR, using the joining primer, GAAATTCTGTTTTTTTAATCTGGAGACATTGTAGCC-3′ (ref. 32). Abbreviations: LTR, long terminal repeat; SV40 Ori, simian virus 40 origin of replication.

TABLE 2 Toxin presentation is not MHC class II allele-specific

Antigen-presenting cells	T cells	None	SEA	SEB	SEC1	SEC2	SEC3	SED
		Units ml^{-1} IL-2 produced in response to *S. aureus* toxins						
B10·A(5R) (I-A^{b}I-E^b)*	hVβ13.1-1	—†	—	—	—	—	—	—
	hVβ13.2-1	—	—	—	—	567	389	—
	hVβ13.1D2-1	—	—	—	—	712	311	26
B10·BR (I-A^{k}I-E^k)	hVβ13.1-1	—	—	—	—	—	—	—
	hVβ13.2-1	—	—	—	—	138	99	—
	hVβ13.1D2-1	—	—	—	—	540	157	—
B10·D2 (I-A^{d}I-E^d)	hVβ13.1-1	—	—	—	—	—	—	—
	hVβ13.2-1	—	—	—	—	529	246	—
	hVβ13.1D2-1	—	—	—	—	1,610	730	—
B10·Q (I-A^q)	hVβ13.1-1	—	—	—	—	—	—	—
	hVβ13.2-1	—	—	—	—	186	79	—
	hVβ13.1D2-1	—	41	—	—	3,013	1,729	—
B10·HTT (I-A^{s}I-E^s)	hVβ13.1-1	—	—	—	—	—	—	—
	hVβ13.2-1	—	45	—	—	363	418	22
	hVβ13.1D2-1	—	76	—	36	4,975	1,814	74

The three T-cell hybridomas described in Table 1 were tested for IL-2 production in response to toxins presented by spleen cells from different strains of mice. Spleen cells were prepared by treatment with anti-T serum, followed by guinea pig complement treatment and irradiation (1,000 rad). Background IL-2 production from the toxin-treated, T-cell-depleted spleen cells alone (10–60 U ml^{-1}) were subtracted from that obtained with the T-cell hybridomas present.

* Haplotype of class II molecules are indicated in parentheses.

† <20 U IL-2 ml^{-1} indicated by —.

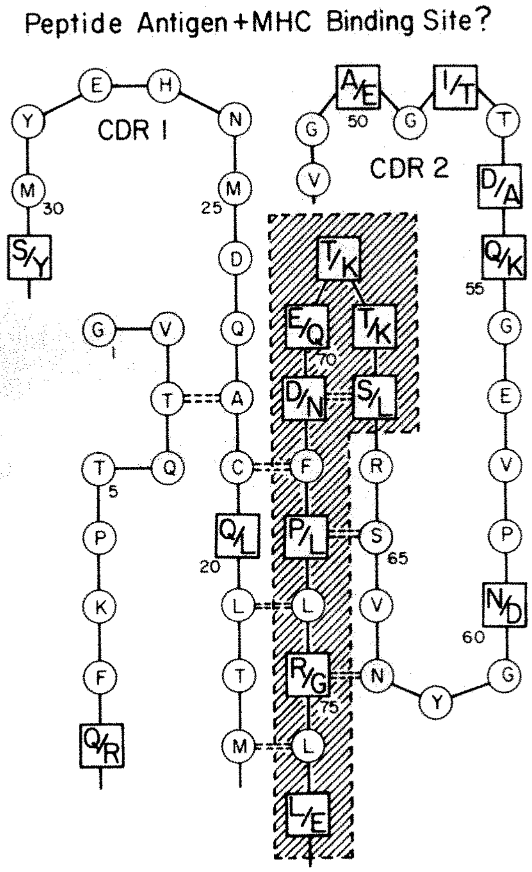

FIG. 2 Alignment of the amino acids (single-letter code) of hVβ13.1 and hVβ13.2 to the predicted structure of the TCR. The structure of a portion of Vβ shown here was adapted from a model of the TCR V domains by Chothia *et al.*[24] (see Fig. 3 in ref. 24). The amino acids differing in hVβ13.1 and hVβ13.2 are shown at their predicted positions as X/Y within squares, where X is amino-acid residue of hVβ13.1 and Y is hVβ13.2. The residues in common between hVβ13.1 and hVβ13.2 are shown in circles. The polypeptide hβ13.1D2 has the amino-acid residues of hVβ13.2 in the hatched box region and those of hVβ13.1 in the rest of TCR Vβ. The sites labelled CDR1 and CDR2 are predicted along with CDR3 (Dβ–Jβ, not shown) to form the portion of Vβ responsible for peptide antigen–MHC recognition.

T cells during thymic selection (P.M., M. Blackman, K. Choi and J.K., manuscript in preparation).

Finally, the human *Vβ*-expressing transfectants produced in these studies offer all the advantages of mouse T-cell hybridomas in that they are easy to maintain and produce in large quantities. The fact that all the components of TCR except Vβ are from mouse, renders this system suitable for the generation of mouse antibodies against particular human Vβ elements. In fact we have produced sero-positive animals by immunizing mice with these cell lines (Y.C., J. Lafferty, J. White, B. Kotzin, P.M. and J.K., manuscript in preparation). It should also be easy to modify this protocol, using an appropriate vector/recipient-cell system, to generate murine cells expressing human Vα elements. □

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The $\alpha 1$ domain of the HLA-DR molecule is essential for high-affinity binding of the toxic shock syndrome toxin-1

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SEVERAL exoproteins from the bacterium *Staphylococcus aureus* are highly potent polyclonal activators of T cells in the presence of cells bearing class II antigens of the major histocompatibility complex (MHC)[1-3]. These toxins, including the toxic shock syndrome toxin (TSST-1), act at nanomolar concentrations, bind directly to class II molecules, and do not require the processing typical of nominal antigen[3-7]. Each toxin is capable of stimulating a subpopulation of peripheral T lymphocytes bearing particular Vβ sequences as part of their $\alpha\beta$ T-cell receptors[8,9]. It is not known how these so-called 'superantigens' bind to class II and how this binding stimulates T cells. In this study, the different affinities of TSST-1 for human class II molecules DR and DP were exploited to define the region of a class II molecule necessary for high-affinity binding. Using chimaeric α- and β-chains of DR and DP expressed at the surface of transfected murine fibroblasts and a binding assay with TSST-1, it was shown that the $\alpha 1$ domain of DR is essential for high-affinity binding, and further that TSST-1 binding did not prevent subsequent binding of a DR-restricted antigenic peptide. This is compatible with a model of superantigen making external contacts with both class II and T cell receptor, and suggests that the Vβ portion of the T-cell receptor interacts with the nonpolymorphic α-chain of DR.

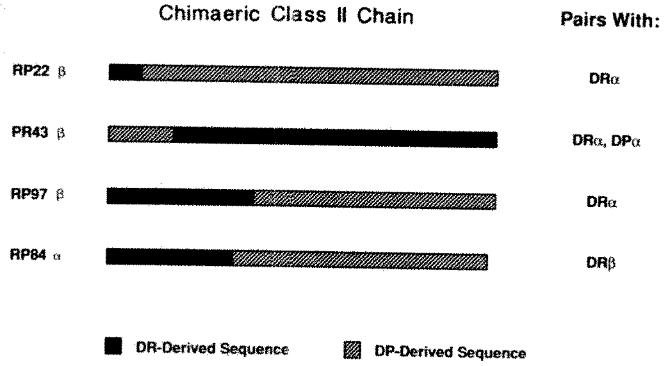

FIG. 1 Schematic diagram of chimaeric class II α- and β-chains. Chimaeric cDNAs having sequences derived from both DR (solid) and DP (hatched) α- or β-chains were constructed for subcloning into a polyoma-based eukaryotic expression vector. The ability of each chimaeric chain to pair, and be expressed with wild-type chains from either isotype is indicated.

METHODS. The hybrid DR–DP β-chain constructs characterized previously were also used in this study[12]. A chimaeric cDNA containing sequences derived from the $\alpha 1$ domain of DR and the $\alpha 2$ domain of DP was constructed using the technique of 'synthesis by overlap extension', and will be described in detail elsewhere (D.R.K., manuscript in preparation). Murine fibroblasts (L cells) that stably express either the DR or DP α-chain or the DR β-chain were transfected with the replicating vector having a wild-type or chimaeric partner chain cDNA, using DEAE-dextran to facilitate DNA uptake as described. The cells were cultured in 10 mM sodium butyrate for 24 h before analysis to maximize transfected class II gene expression. After transfection (48 h), the cells were analysed for ability to bind iodinated TSST-1 (see Fig. 2 legend). A portion of the cells was stained with the monoclonal antibody SG465 (a gift of Dr S. Goyert)[20] and analysed by microcytofluorimetry for class II expression.

Functional assays for T-cell activation reveal that all three expressed isotypes of human class II, HLA-DR, -DQ, and -DP, are capable of interaction with staphylococcal toxins[5,10]. Several groups have demonstrated the direct, high affinity (dissociation constant $K_d \approx 10^{-9}$ M) binding of toxins to class II molecules[4,6,7,11]. All isotypes and allotypes of human class II tested so far interact with at least one of the staphylococcal toxins, but there are several differences. For example, [^{125}I] TSST-1 binding to L cells transfected with HLA-DPw2 or -DPw4 cannot be demonstrated in a radio-ligand binding assay that easily detects binding to HLA-DR and -DQ alleles[10]. By contrast, T-cell stimulation by TSST-1 in the presence of DP-positive L cells was comparable to that seen with the other isotypes. This disparity was felt to represent the greater sensitivity of the cellular assay compared with direct binding assays.

L cells expressing either the DR or DP α-chain were transiently transfected with complementary DNAs for chimaeric β-chains[12]. The cells were simultaneously tested for the ability to bind the staphylococcal toxin, TSST-1, and for class II expression. The TSST-1 binding assay has been shown to be dependent on the presence of class II molecules on the surface of the L cells and is completely inhibitable by the presence of excess unlabelled TSST-1 (ref. 6). This assay detects binding to various alleles of HLA-DR in stably transfected L cells over a wide range of levels of class II expression[10]. In addition to the wild-type DR β-chain, three chimaeric β-chains were each expressed in conjunction with the DR α-chain (Fig. 1)[12]. The chimaeras RP22 and RP97 have DR β-chain sequence for the first 22 and 97 amino acids, respectively, and DP β-chain sequence for the remainder of the molecule. The chimaera PR43 has DP β-chain sequence for the first 43 amino acids and DR sequence for the rest of the β-chain. Taken together, the portion of the β-chain sequence derived from DP spans the entire β-chain. Cells bearing class II molecules consisting of any of

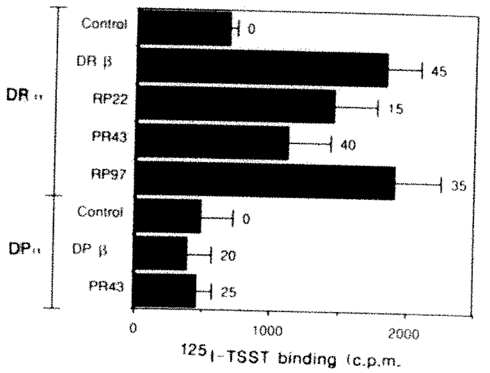

FIG. 2 Binding of iodinated TSST-1 to L cells expressing class II molecules having wild-type or chimaeric β-chains. Cells expressing either the DR or DP α-chains were transfected with either the wild-type isotypic β-chain or the indicated chimaeras. After transfection (48 h), 10^5 live cells were tested for their ability to bind [^{125}I]TSST-1. Controls for each α-chain were transfections with the β-chain of the non-homologous isotype, which is expressed intracellularly, but does not pair with the α-chain and does not appear at the cell surface. The mean and standard errors of triplicate determinations from one representative experiment are shown. The percentage of cells in each transfection that react with an anti-class II monoclonal antibody (SG465) is given to the right of each bar.

METHOD. Toxin-binding was assessed by incubating 10^5 live cells in the presence of 10^{-8} M [^{125}I]TSST-1 for 1.5 h on ice[6]. The cells were layered over a cushion of mixed phthalate oils and centrifuged in a 400 μl polypropylene tube. The tips of the tubes containing the cell pellets were then placed in a gamma counter. The values from triplicate determinations were averaged.

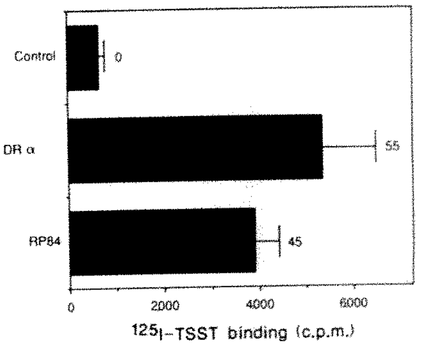

FIG. 3 Binding of iodinated TSST-1 to L cells expressing class II molecules with a chimaeric α-chain. L cells that stably express the DR1 β-chain were transfected with cDNAs for the wild-type or PR84 α-chain and analysed for their ability to bind TSST-1 as above. Control cells were transfected with the DP α-chain, which is expressed intracellularly but does not form $\alpha\beta$ dimers at the cell surface (C.L.T., unpublished data). The percentage of SG465-positive cells is given to the right of each bar.

these chimaeric β-chains paired with DRα are capable of binding TSST-1 (Fig. 2). The percentage of cells stained by the anti-class II antibody, SG465, varies from 15–45%. The amount of toxin bound per 10^5 cells varied from chimaera to chimaera, but was reproducible in separate experiments, and not absolutely related to the level of class II expression. Therefore, the sequences necessary for the high affinity (DR-like) binding of toxin are not located in the β-chain, although the β-chain may exert an effect on the relative efficiency of this interaction.

One of these chimaeric β-chains (PR43) is also expressed with the DP α-chain. TSST-1 did not bind to cells transfected with the DP α-chain and PR43, although high levels of class II were expressed at the cell surface. As expected, cells transiently expressing the wild-type DPw2 molecule did not bind the toxin. Another β-chain chimaera, PR59, which is expressed with both DR and DP α-chains[12] was also tested for TSST-1 binding (not shown). As with the PR43 β-chain, toxin binding was only observed when PR59 was paired with the DR α-chain, and not when it was paired with the DP α-chain. This confirms the finding that it is the α-chain of DR that is required for the high affinity binding to TSST-1.

The cDNA coding for an α-chain having an α1 domain derived from DR and an α2 domain derived from DP was subcloned into the polyoma-based expression vector, R3CPy-II and transfected into murine L-cells that were stably expressing the DR β-chain. This chimaeric α-chain, RP84, pairs very efficiently with DRβ and is expressed at high levels at the cell surface, but is not expressed at the cell surface with the DP β-chain (data not shown). Both the cells expressing the wild-type DR1 molecules and those expressing the chimaeric α-chain bind TSST-1 (Fig. 3). This observation, along with the data presented above, demonstrate that the interaction of TSST-1 with HLA-DR requires sequences present in the α1 or amino-terminal portion of the α-chain. These data do not prove that the toxin is binding directly to α1 sequences, but that is the most straightforward interpretation. Binding may involve a combination of α- and β-chain sequences, or may even be to the β-chain alone, but strictly requires the presence of the DR α-chain for proper conformation. Repeated attempts to demonstrate binding of TSST-1 to α- or β-chains isolated by SDS-PAGE have been unsuccessful, suggesting that the toxin-binding epitope has either a conformational or combinatorial requirement.

It is not known whether the toxin stimulates T cells by inducing a conformational change in class II that is then recognized by the T-cell receptor (TCR), or alternatively by simultaneously contacting both class II and TCR. As either intact TSST-1 (relative molecular mass 22,000; M_r 22K) or a 14K fragment are required for functional activity[13,14], it is unlikely to bind within the postulated peptide-binding 'groove' formed by the α1 and β1 domains of class II[15], although this possibility exists. Additionally, TSST-1 may block access to the class II site by allostearic effects. To investigate this, class II-bearing cells were incubated in the presence of a biotinylated analogue of the main DR1-restricted antigenic peptide from influenza haemagglutinin [LCB-HA(307–319)] (ref. 16) with or without a prior incubation in saturating amounts of TSST-1. The cells were then stained with fluorescein isothiocyanate-conjugated avidin and rabbit anti-TSST (plus phycoerythrin labelled goat anti-rabbit immunoglobulin) and analysed by microcytofluorimetry. The B-cell line 45.1 expresses the HLA-DR1, -DQw1 and DPw2

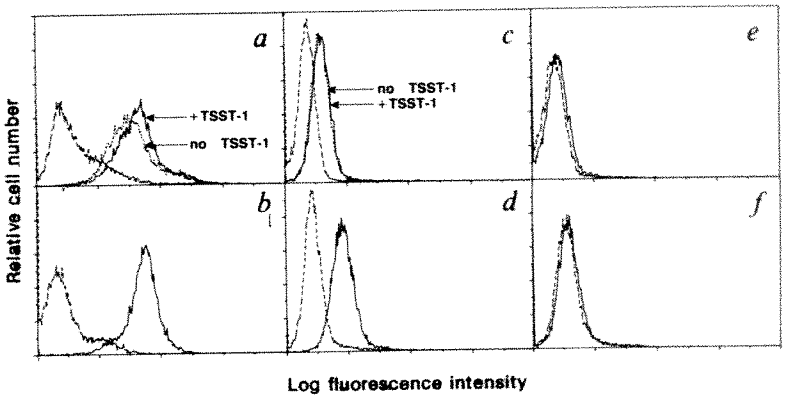

FIG. 4 Binding of an antigenic peptide is unaffected by TSST-1. The B-cell line 45.1 and L-cell transfectants expressing DR1 and DPw2 were incubated in the presence of the synthetic peptide LCB-HA(307–319), with or without prior incubation with TSST-1. The cells were then stained as described below and analysed by microcytofluorimetry. *a*, *c*, Control staining (----); binding of LCB-HA(307–319) alone (· · · ·); binding of LCB-HA(307–319) after binding of TSST-1 (——) to 45.1 (*a*) and DAP-DR1 (*c*). *e*, Control (----); binding of TSST-1 (——) to 45.1 (*b*), DAP-DR1 (*d*), and DAP-DPw2 (*f*).

METHODS. The peptide LCB-HA(307–319) was obtained from Dr J. Rothbard, (ImmuLogic Pharmaceutical Corp., Palo Alto, California). It has the sequence (N- to C-terminal): PRYVRQNTLRLAT (single-letter amino-acid code) which corresponds to the substitution of arginine for lysine at positions 308, 311, and 316 in the native haemagglutinin sequence to prevent biotinylation of ε amino groups. The peptide was coupled to NHS-LC-biotin (Pierce) through the α-amino group. This peptide competes effectively for the native HA(307–319) in both direct binding and antigen presentation assays[16]. The hemizygous B-cell line, 45.1, has the HLA-D region haplotype DR1, DPw2, DQw1 (ref. 17). DAP-DR1 and DPw2 are clones of L-cell stably transfected with cDNAs for the α- and β-chains of DR1 and DPw2, respectively. Cells were incubated 5×10^5 for 1 h at 4 °C, in either 100 μl of complete medium or 100 μl of a 10 μg ml^{-1} solution of TSST-1 ($\sim4\times10^{-7}$ M) diluted in complete medium. The cells were then diluted with either 100 μl of complete medium or a 200 mM solution of LCB-HA(307–319). The cells were incubated at 37 °C for 6 h, then washed and stained as follows: FITC–avidin D (1:100, Vector Laboratories), wash, biotinylated goat anti-avidin (1:100, Vector Laboratories) plus rabbit anti-TSST-1 (1:100, Toxin Technologies, Madison, Wisconsin), wash, FITC-avidin again, plus phycoerythrin-labelled goat anti-rabbit Ig (1:100, Southern Biotechnology Associates, Birmingham, Alabama). After a final wash, the cells were analysed by two-colour microcytofluorimetry on the FACScan (Becton-Dickinson, Mountain View, California).

molecules on its surface[17]. These cells bound equivalent amounts of the peptide regardless of the presence of TSST-1 (Fig. 4*a*). That TSST-1 was actually bound to these cells is demonstrated in Fig. 4*b*. The data are shown as separate fluorescence histograms for clarity; two-colour fluorescence contour plots revealed a single homogeneous population of cells positive for both peptide and TSST-1 binding. Figure 4*c* demonstrates similar findings for a stable L-cell transfectant expressing DR1. The quantity of class II on these cells is about one-tenth that of the B cells. The level of peptide binding was correspondingly lower. Again, pre-incubation of the cells with TSST-1 did not affect the level of peptide binding. The binding of TSST-1 to these cells is shown in Fig. 4*d*. An L-cell transfectant expressing equivalent amounts of DPw2 did not bind either the biotinylated peptide or TSST-1 (Fig. 4*e*, *f*).

It has been estimated that ~1% of the surface DR molecules will bind the haemagglutinin peptide under the conditions used[16]. Presumably, the toxin is unable to distinguish those class II molecules that will eventually bind the biotinylated peptide from those that do not. The simultaneous binding of TSST-1 and peptide to the same DR molecule is then most consistent with the model of toxin contacting both the class II and the TCR outside of the peptide-binding site.

There is some evidence for a functional interaction between the α-chain of class II and the β-chain of the T cell receptor. The maximal response of murine T cells to a mitogen from *Mycoplasma arthriditis*, which is predominantly by cells bearing Vβ6 and Vβ8 TCRs, requires the presence of the I-E α-chain[18]. L cells expressing either the Eα-*E*β or Eα-Aβ murine class II molecules were far better stimulators of purified T cells in the presence of mycoplasmal mitogen than were fibroblasts expressing Aα-Aβ. Also, the expression of an Eα transgene in H-2^b mice that do not normally express Eα leads to the ability of the mitogen to stimulate splenocytes *in vitro*. This is consistent with the hypothesis that this mitogen requires the presence of Eα for binding to class II. In addition, Berg, *et al.*[19] have studied the selection of the T-cell repertoire in TCR transgenic mice. Mice that express transgenes for particular TCR α- and β-chains have strict requirements for the class II haplotypes that will lead to positive selection of the transgenic TCR. However, mice that express the transgenic TCR β-chain in association with a variety of endogenous TCR α-chains are selected by several I-E haplotypes (all of which share a nonpolymorphic class II α-chain), suggesting that the TCR Vβ region interacts with the class II α-chain. These data argue for a functional, if not physical, interaction between the α-chain of I-E and the Vβ region of the TCR. The finding that the high-affinity binding of TSST-1 to HLA-DR requires the α1 domain is consistent with this hypothesis, and suggests that there are contact points between the DR α-chain and the Vβ domain of the TCR. □

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Empty MHC class I molecules come out in the cold

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MAJOR histocompatibility complex (MHC) class I molecules present antigen by transporting peptides from intracellularly degraded proteins to the cell surface for scrutiny by cytotoxic T cells. Recent work suggests that peptide binding may be required for efficient assembly and intracellular transport of MHC class I molecules[1], but it is not clear whether class I molecules can ever assemble in the absence of peptide. We report here that culture of the murine lymphoma mutant cell line RMA-S at reduced temperature (19–33 °C) promotes assembly, and results in a high level of cell surface expression of H-2/β_2-microglobulin complexes that do not present endogenous antigens, and are labile at 37 °C. They can be stabilized at 37 °C by exposure to specific peptides known to interact with H-2K^b or D^b. Our findings suggest that, in the absence of peptides, class I molecules can assemble but are unstable at body temperature. The induction of such molecules at reduced temperature opens new ways to analyse the nature of MHC class I peptide interactions at the cell surface.

A mouse mutant lymphoma cell line, RMA-S, expresses at the cell surface <10% of the amount of class I molecules compared with RMA mutagenized but unselected control line[2,3]—and is unable to present endogenous antigens[1,4]. Rates of synthesis of class I heavy and light chains are normal in RMA-S, and analysis of RMA-S-L-cell hybrids excludes the possibility that the defect involves a mutation in the H-2 heavy or light chain of RMA-S[5].

In an experiment originally designed to examine the contribution of the various protein breakdown routes[6] to reduced surface expression in RMA-S, cells were pulse-labelled at 37 °C and chased at 26 °C. At this temperature, essentially no breakdown of class I molecules of the RMA wild-type line occurred, and glycan modifications were slower compared with chase at 37 °C (Fig. 1*a*). Breakdown of class I molecules was also decreased at 26 °C for RMA-S but, surprisingly, extensive modifications of *N*-linked glycans were observed (Fig. 1*a*).

Culture of RMA-S at temperatures ranging from 8–35 °C (optimum 23–31 °C) also led to a marked increase in the expression of K^b and D^b at the cell surface (Fig. 2*a*). Expression increased linearly with time (shown for 22 °C), up to 28 h (Fig. 2*b*). Culture of RMA-S at 26 °C for 24 h (notation, RMA-S (26 °C)) was adopted as the standard treatment, unless stated otherwise. The class I molecules that appear at the cell surface at low temperature seem to be folded correctly, and are associated with β_2-microglobulin. For both D^b and K^b, a parallel increase in the binding of monoclonal antibodies to epitopes on the α1, α2 or α3 domains of the heavy chain, as well as to β_2-microglobulin was observed (Fig. 2*c*). Comparison of surface-labelled material of RMA-S (26 °C) and RMA-S (37 °C) immunoprecipitated with either anti-H-2^b serum or the conformation-independent anti-K^b exon 8 antibody (a gift from Dr B. Barber) reveals the existence of class I molecules on RMA-S (37 °C) that are not recognized by the anti-H-2^b serum (possibly reflecting the population of H-2 molecules that can be stabilized by addition of peptide at 37 °C). But there is a significant increase in the amount of material recovered with both antisera on culture at reduced temperature (data not shown). The effect of low temperature on class I expression on RMA-S differs from the effect of peptides added at 37 °C[1]. Low temperature increases the expression of both D^b and K^b, whereas peptides have predominantly allele-specific effects[1]. The two effects are additive during the first 6 h of incubation at 26 °C (data not shown). The effect of low temperature on class I expression was also observed, but to a lesser extent, on RMA under identical conditions (Fig. 2*b*).

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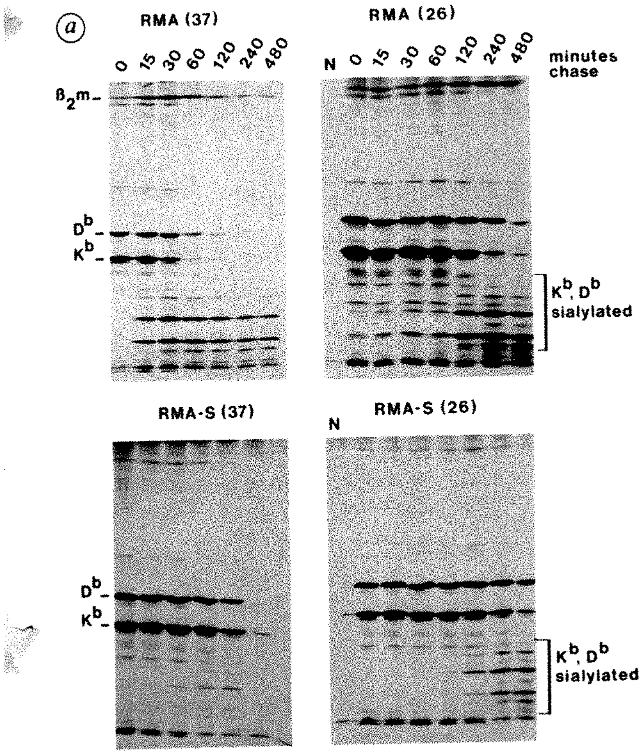
RMA-S has been reported to be incapable of presenting internally derived antigens to H-2-restricted cytotoxic T lymphocytes (CTL) specific for viral[1], minor histocompatibility or tumour antigens[4]. RMA-S (26 °C) remained resistant to H-2^b-restricted CTL directed against minor histocompatibility antigens (Fig. 3*a*), despite a 5- to 10-fold increase in H-2K^b and D^b expression at the cell surface measured in parallel by cytofluorimetry (data not shown). Similarly, the RMA-S (26 °C) cells remained resistant to a H-2D^b-restricted influenza-specific CTL clone after virus infection at 26 °C (Fig. 3*b*). But when tested with a H-2D^b-restricted peptide, NP 366-379[1], RMA-S (26 °C) was 100-fold more susceptible to lysis than RMA-S (37 °C) in terms of the peptide concentration required to induce 50% killing (Fig. 3*c*). Although RMA-S (26 °C) had half the H-2D^b expression of RMA (37 °C), RMA-S (26 °C) could be sensitized at a far lower concentration of peptide than RMA (37 °C). Note that the peptide titration curve shifted also for RMA (26 °C).

We propose that the class I molecules exported to the cell surface at 26 °C in RMA-S lack peptide in their antigen-binding pocket. Our previous experiments suggested that peptide is required for assembly and stabilization of class I molecules at 37 °C in RMA-S[1]. To test whether the class I molecules induced in RMA-S (26 °C) were unstable at physiological temperature,



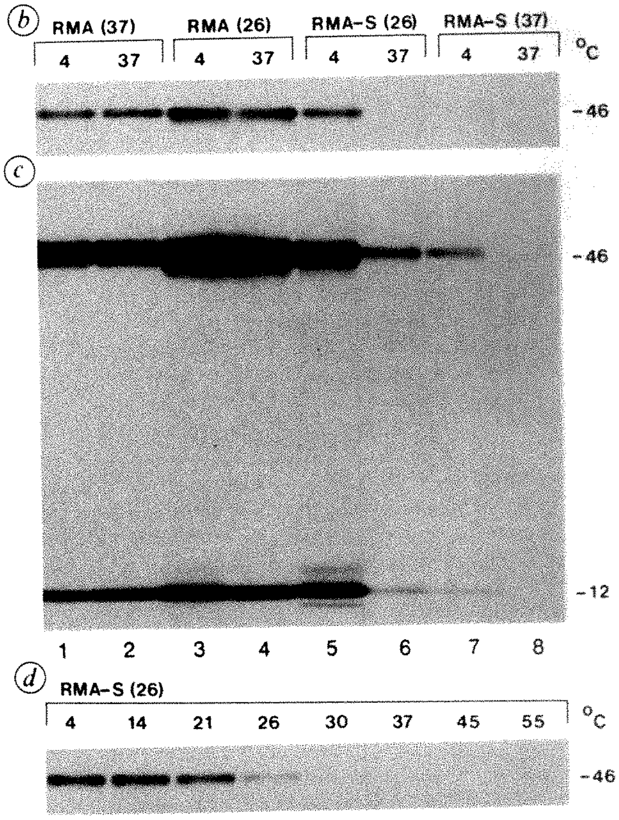


FIG. 1 Post-translational modifications and stability of class I molecules induced at low temperature. *a*, RMA and RMA-S were cultured and metabolically labelled for 10 min at 37 °C, chased for the times indicated at either 26 °C or 37 °C, and lysed. Class I molecules were immunoprecipitated and analysed on 1D-IEF gels. The position of β_2-microglobulin (β_2m) and nonsialylated H-2K^b and D^b are indicated on the left, sialylated forms of K^b and D^b by the brackets on the right. Class I molecules showed a significantly prolonged half life at 26 °C in comparison with 37 °C for both RMA and RMA-S. Importantly, the extent of terminal glycan modifications (sialylation), indicating transport through the trans-Golgi, was higher in RMA-S chased at 26 °C than at 37 °C. *b*, *c*, RMA and RMA-S were cultured at either 37 °C or 26 °C, surface-labelled by iodination and lysed. Lysates were incubated at the temperatures indicated. Class I molecules were then immunoprecipitated and analysed by SDS-PAGE. Note the disappearance of anti-H-2 reactive material of RMA-S (26 °C) on incubation of the lysate at 37 °C (only the heavy chain region of the gel is shown in *b*). The small amounts of H-2 that are expressed on the surface of RMA-S (37 °C) exhibit similar thermolability (*c* is a longer exposure of *b*). *d*, Incubation of RMA-S (26 °C) lysates was performed at different temperatures, as indicated. Note the reduction in immunoreactive H-2 class I molecules after incubation at temperatures higher than 26 °C.

METHODS. *a*, Ten million cells were pulse-labelled with 500 μCi[^{35}S]methionine (1,200 Ci mol^{-1}, Amersham) for 10 min at 37 °C and chased in the presence of excess of cold methionine for different periods at either 26 °C or 37 °C (ref. 14). After lysis, free β_2m was removed by immunoprecipitation using an anti-β_2m serum (K297, ref. 15). Specific immunoprecipitation used a rabbit anti-mouse H-2 serum (a gift from L. Rask, Uppsala; ref. 16 and S. Nathenson, New York) and analysed on 1D-IEF as described (ref. 17 and H-G.L., M. Oudshoorn-Snoek, M. G. Masucci and H.L.P., manuscript submitted). The anode is at the bottom. N, immunoprecipitates prepared with normal mouse serum as control. *b*-*d*, Cells were cultured for 48 h at 26 °C or 37 °C in RPMI-1440 medium, fetal calf serum (FCS). Five million cells were surface-labelled by lactoperoxidase-catalysed iodination as described[18]. Cells were lysed on ice in 1 ml lysis buffer (1% Triton X-100, 140 mM NaCl, 10 mM Tris buffer, pH 7.8, 1 mM phenylmethylsulphonyl fluoride, 1 μg ml^{-1} trypsin inhibitor, 1 μg ml^{-1} leupeptin, 30 mU of trypsin inhibitor ml^{-1} aprotinin (Sigma)). The lysates were incubated at the temperature indicated for 1 h and transferred to 0 °C. H-2 class I were immunoprecipitated with rabbit anti-H-2 serum (see above) as described[17], and analysed by SDS-PAGE on a 12% gel. Relative molecular mass standards (M_r ×10^{-3}) are to the right.

H-2 expression was examined after shifting the cells to 37 °C. Brefeldin A (BFA) was added to prevent further transport of class I molecules from the endoplasmic reticulum to the cell surface[7,8]. Within 1 h, more than half the D^b class I molecules induced at 26 °C had disappeared from the cell surface (Fig. 4*a*). After 4 h at 37 °C only trace amounts of induced K^b and D^b molecules remained (Fig. 4*b*). A similar decrease was observed for K^b and D^b in BFA-treated RMA-S (37 °C) cells (Fig. 4*a*, 4*b*), in agreement with the rapid decay of biosynthetically labelled class I molecules in RMA-S (37 °C). By contrast, more than 80% of class I molecules was still detectable at the surface after 2 h, in BFA-treated RMA (37 °C) cells and their halflife was >6 h as measured by cytofluorimetry (data not shown).

Significant amounts of surface-iodinated H-2 class I molecules, comparable to the amounts seen in RMA (37 °C), could be immunoprecipitated from RMA-S (26 °C) (compare lanes 1 and 5, Fig. 1*b*, *c*). When lysates were incubated at 37 °C, a complete loss of immunoreactive class I molecules of RMA-S (26 °C) or (37 °C) was observed (compare lanes 5 and 6; Fig. 1*b*–*d*). For RMA-S lysates, but not for RMA lysates, there was a sharp drop in quantity of H-2 recovered, with increasing temperature (Fig. 1*d*). The loss of H-2 class I molecules in the lysates from RMA-S was not due to proteolysis, as judged by the continued presence of H-2K^b and D^b heavy chains revealed by two-dimensional isoelectric focusing (IEF)/SDS-PAGE analysis of the total lysate (data not shown).

If the class I complexes induced at the RMA-S cell surface at low temperature are unstable at 37 °C because they are devoid of peptides, addition of class I-binding peptides to BFA-treated cells before transfer to 37 °C, should stabilize them. In accordance with their known restriction specification, this was indeed the observed result for epitopes presented in the context of H-2K^b and D^b (Fig. 4*b*, *c*), at concentrations of peptide, previously shown to restore H-2 expression in RMA-S (37 °C). This peptide-mediated stabilization could be confirmed by biochemical analysis of surface-labelled RMA-S (26 °C)[9].

In summary, culture of RMA-S at low temperature induces MHC class I molecules at the cell surface that: (1) undergo post-translational modifications characteristic of passage through the trans-Golgi (Fig. 1*a*); (2) are associated with β_2-microglobulin at the cell surface and express conformational epitopes found in properly folded class I molecules (Figs 1*c* and 2); (3) are unstable at 37 °C (Figs 1*b*–*d* and 4*a*–*b*); (4) fail to present internally derived antigens to CTL (Fig. 3*a*, *b*); (5) present exogenous peptides more efficiently than class I molecules of the wild-type line cultured at 37 °C (Fig. 3*c*), and (6) are stabilized by exogenous peptides in an allele-specific manner (Fig. 4*b*, *c*). The most simple interpretation is that MHC class I molecules devoid of internally derived peptides can reach the cell surface at the reduced temperature.

We propose the following explanation for our findings. Under normal conditions, the MHC class I subunits will combine with peptide early in biosynthesis. If they fail to do so, a heterodimer may fail to form or, if devoid of peptide, have a high probability of dissociating or displaying a grossly altered conformation at 37 °C. We surmize that such 'empty', aberrantly folded heavy chains would not be transported further and be rerouted or degraded. That fraction of class I/β_2-microglobulin heterodimers that reaches the cell surface empty at 37 °C can be stabilized by a suitable peptide supplied during experimental procedures. The intrinsic instability of empty class I molecules can be overcome not only by addition of peptide, but also by reducing the temperature. The affinity of the class I heavy chain for β_2-microglobulin is thus sufficiently high at the reduced temperature to allow an increase in net transport of the complex to the cell surface in the absence of peptide.

The findings reported here underscore the role for peptide in maintaining structural integrity of class I molecules, but are consistent with the possibility that much of the increase in H-2

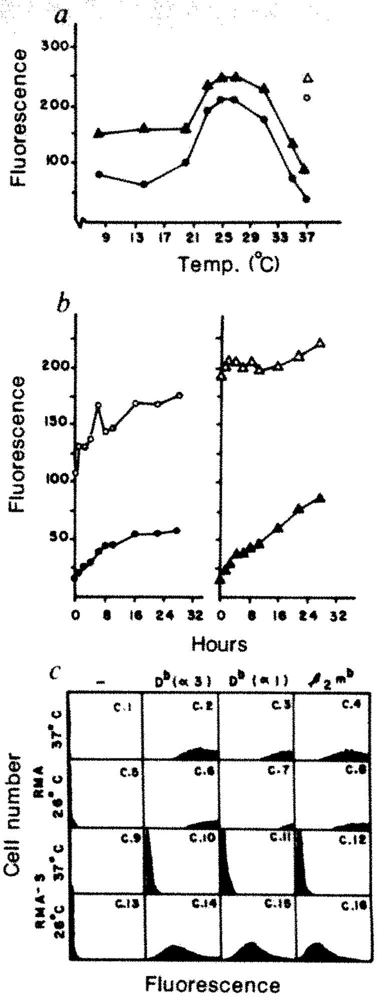


FIG. 2 Induction of cell surface class I molecules at low temperature. *a*, Staining with monoclonal antibodies against K^b (28-13-3S; ref. 19) and D^b (28-14-8S; ref. 20–22) at the cell surface of RMA-S cells cultured for 24 h at different temperatures. Quantitation of K^b and D^b expression on the control line RMA cultured at 37 °C is indicated for comparison. ●, RMA-S anti-K^b; ▲, RMA-S anti-D^b; ○, RMA anti-K^b; △, RMA anti-D^b. On average, in a series of 10 experiments, K^b and D^b expression increased to a level corresponding to 40–55% of RMA (37 °C). In occasional experiments the level of expression reached that of RMA (37 °C). *b*, Kinetics of class I increase at the cell surface measured with monoclonal antibodies against H-2K^b (28-13-3S) and D^b (28-14-8S) at 22 °C on RMA-S and RMA. ●, RMA-S anti-K^b; ▲, RMA-S anti-D^b; ○, RMA anti-K^b; △, RMA anti-D^b. *c*, H-2D^b induced at low temperature is associated with β_2m and expresses a conformation-dependent epitope on the $\alpha1$ domain. Cell surface staining with monoclonal antibodies 22-14-8S (D^b, $\alpha3$; charts C.2, C.6, C.10 and C.14), B22.249 (D^b, $\alpha1$; charts C.3, C.7, C.11, C.15; ref. 21–23) and mc-β_2m-B ($\beta_2$mb allele; charts C.4, C.8, C.12, C.14) of RMA (charts C.1–C.8) and RMA-S (charts C.9–C.14) cultured for 24 h at 26 °C or continuously at 37 °C. Charts C.1, C.5, C.9 and C.13 show background staining with no first (specific) antibody (–). Similar results were obtained for K^b with the antibodies Y3 ($\alpha2$, ref. 25) and S19.8 (ref. 26) (data not shown).

METHODS. All cells were cultured in RPMI-1440, 10% FCS supplemented with antibiotics. Cells were removed from 37 °C (5% CO_2), cultured (10^6 cells ml^{-1}) in tissue-culture flasks (Falcon, 50 ml) with tightened cap, placed in a heat-adjustable water bath for 24 h at a given temperature unless noted otherwise. Culture at temperatures <8 °C for 24 h or more, usually reduced viability of cells. For cytofluorometry, 10^6 cells were incubated with 0.1 ml anti-class I monoclonal antibody tissue culture supernatant for 30 min on ice, washed twice in PBS, and then incubated with 0.1 ml fluorescein isothiocyanate (FITC)-conjugated rabbit anti-mouse immunoglobulin (Z109; DAKO, Copenhagen, Denmark) on ice for 30 min, washed twice in PBS, fixed in 1% formaldehyde and analysed on a FACS IV cell sorter. Results from *a* and *b* derived from mean linear fluorescence values obtained by FACS analysis. Monoclonal antibodies 28-14-8S and 28-13-3S were obtained from ATCC (Rockville, MD), B22.249 was a gift from G. J. Hammerling, mc-β_2m-B (ref. 27) was a gift from E. A. Boyse.

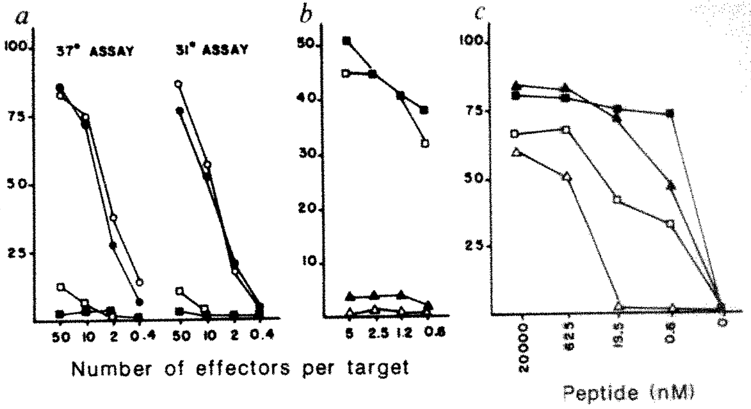


FIG. 3 Sensitivity to cytotoxic effector cells after induction of class I molecules at low temperature on RMA and RMA-S. *a*, RMA-S(26 °C) cells are not recognized by CTL specific for minor histocompatibility antigens in ^{51}Cr release assay at 37 °C (left chart) or at 31 °C (right chart). Target cells were: , RMA (37 °C); ○, RMA (26 °C); ■, RMA-S (37 °C); □, RMA-S (26 °C). Note that this resistance was also observed if the cytotoxicity assay was at 31 °C (Fig. 3*a*), that is, within the temperature range permissive for high levels of cell surface expression of class I molecules (Fig. 2*a*). *b*, Low temperature treated RMA-S cells infected with a recombinant vaccinia that expresses a rapidly degraded C-terminal fragment of influenza NP (IMP-VAC, ref. 28) are also not recognized by the NP-specific CTL clone, F5. Target cells were: □, RMA (37 °C) infected with IMP-VAC; ■, RMA (26 °C) infected with IMP-VAC; △, RMA-S (37 °C) infected with IMP-VAC; ▲, RMA-S (26 °C) infected with IMP-VAC. Uninfected cells were not lysed above 1% after treatment at either temperature (not shown). *c*, Recognition by clone F5 of RMA and RMA-S after incubation at 26 °C or 37 °C and exposure to decreasing concentrations of peptide NP 366–379 (ref. 1). △, RMA-S (37 °C); ▲, RMA-S (26 °C); □, RMA (37 °C); ■, RMA (26 °C). The CTL clone F5 was used at a killer/target ratio of 5/1 throughout.

METHODS. CTL in *a* were generated by secondary mixed lymphocyte culture across a minor histocompatibility barrier (A.By anti-B6) as described[3]. ^{51}Cr-labelling of targets were performed at 26 °C and 37 °C, respectively. A standard protocol for the ^{51}Cr release assay was used[3]. Clone F5 in *b* was isolated from an influenza virus-infected B6 mouse as described[24]. In *b* it was tested against RMA and RMA-S infected with a recombinant vaccinia that expressed the C-terminal portion of influenza NP (amino acids 327–498) under the control of the vaccinia virus 7.5K promoter[28]. Target cells were incubated at 26 °C or 37 °C for 24 h, collected and infected with 10 plaque-forming units per cell of recombinant vaccinia, and labelled with ^{51}Cr, at the same temperature for 90 min. After washing, the cells were again resuspended at 26 °C or 37 °C for 4 h to allow for synthesis of vaccinia-encoded proteins. Cells were again washed and set up in a 4 h ^{51}Cr release assay as described at 37 °C (ref. 29) with CTL clone F5 at the killer/target cell (K/T) ratios shown. Surface expression of D^b measured with the monoclonal antibody B22.249 (mean fluorescence) measured after incubation at the different temperatures was 20, RMA-S (37 °C); 44, RMA-S (26 °C); 277, RMA (37 °C); 328, RMA (26 °C). In *c*, RMA-S and RMA were collected after 24 h at 26 °C or 37 °C, labelled with ^{51}Cr and incubated with peptide NP 366–379 (ref. 29) at the same temperatures at the concentrations shown for 90 min, before washing and exposing to CTL clone F5 at a K/T ratio of 5:1 for a 4 h ^{51}Cr-release assay at 37 °C. Surface expression of D^b measured with the monoclonal B22.249 after incubation at the different temperatures, but before addition of peptide, was; 9, RMA-S (37 °C); 44, RMA-S (26 °C); 238, RMA (37 °C); 318, RMA (26 °C).

surface expression that is induced by peptide[1], involves stabilization of empty molecules at the cell surface. Indeed, empty class I molecules can efficiently bind peptide in cell lysates[9,10]. The quantity of peptide taken up by RMA-S through endocytosis is sufficient for stabilization of pulse-labelled H-2 molecules in such lysates[10]. There is therefore no evidence in favour of retrograde transport of peptide to the ER where it would induce assembly.

Low-temperature induction of heterodimer assembly in the absence of peptide is a novel phenomenon that opens new ways not only to analyse peptide–MHC class I interactions, but also their role in various functions ascribed to MHC class I

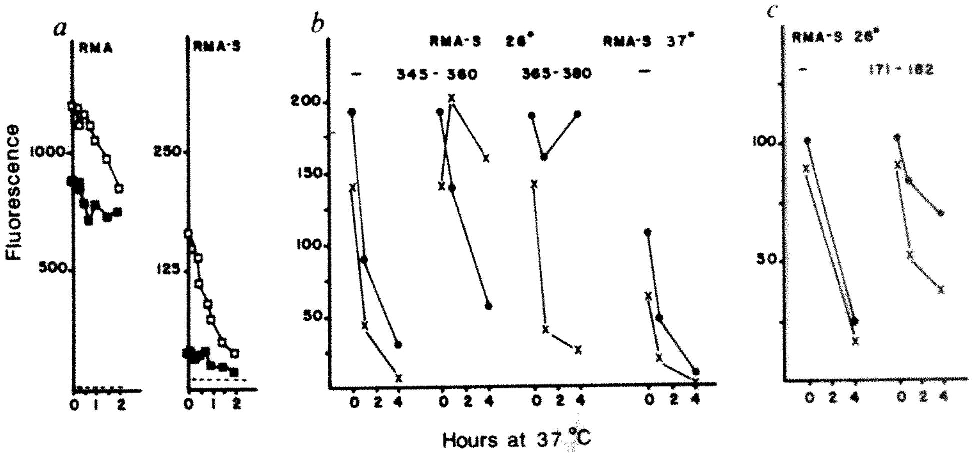


FIG. 4 Class I molecules induced at low temperature on RMA-S are not stable at 37 °C but can be stabilized by peptides added exogenously. The influenza peptide NP 365–380, which is a defined epitope presented by D^b (ref. 29), prevented decay of D^b induced at 26 °C but not of K^b (Fig. 4*b*). The opposite pattern was seen for the peptide NP 345–360, which stabilized K^b but not D^b (Fig. 4*b*). The stabilization lasted for at least 4 h. It was readily observed at a peptide concentration of 10^{-4} M, but was not detectable at concentrations below 10^{-7} M (data not shown). These concentrations of peptide are similar to those required to restore class I cell surface expression in RMA-S at 37 °C (ref. 1). A H–2D^b-derived sequence (residues 171–182), which competes for H–2D^b-restricted presentation of peptide[30] could also stabilize D^b molecules on RMA-S (26 °C) (Fig. 4*c*). A partial but reproducible stabilization of K^b was also observed and is consistent with a low affinity of H–2K^b for the peptide[1]. The decay curves for RMA preincubated at 26 °C and 37 °C converged, suggesting that preincubation at the lower temperature increased the proportion of unstable class 1 molecules. The data are consistent with the existence of two populations of class I molecules with respect to half-life. One induced at 26 °C on both RMA-S and RMA with a half-life of ~1 h at 37 °C, the other found only on RMA and stable at 37 °C with a half-life >6 h. *a*, Decay of H–2D^b on RMA (left chart) and RMA-S (right chart) precultured for 24 h at 26 °C (□) or at 37 °C (■), then incubated at 37 °C in the presence of brefeldin A. Cells were stained with monoclonal B22.249 (D^b, α1) and analysed by FACS (see Fig. 2), at different timepoints after transition to 37 °C. Background staining with secondary fluorescent antibody alone indicated (- - -). *b*, Influenza nucleoprotein-derived peptides prevent decay of induced class I molecules on RMA-S (26 °C). Samples were stained and fixed for FACS analysis (see Fig. 2) after 0, 1, and 4 h at 37 °C, with monoclonals against K^b (×, 28-13-3S); and D^b (●, 28-14-8S). Stabilization generally lasted for at least 4 h. It was readily observed with peptide concentrations of 100 μM or more and was not detected at concentrations below 0.1 μM. *c*, Peptides drived from a conserved region of the H–2D^b molecule (residues 171–182) prevent decay of K^b less efficiently than decay of D^b. Experimental conditions as in *b*.

METHODS. BFA (2 μl, 5 mg ml^{-1} in methanol) was added to a mixture of 0.9 ml of cells in RPMI–FCS and 0.1 ml peptide NP(1934) 365–380 or NP (1968) 345–360, giving a final concentration of 10 μg ml^{-1} BFA and 200 μM peptide. In controls, 0.1 ml medium was added without peptide. The mixture of cells, BFA and peptide was then immediately put at 37 °C (5% CO$_2$).

molecules. Apart from antigen presentation to class I-restricted CTL[1], these functions include interactions with other cell surface molecules[11] and target recognition by alloreactive CTL[4,12] and natural killer cells[2,3,13]. □

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Specific expression of a silk-encoding gene of *Bombyx* in the anterior salivary gland of *Drosophila*

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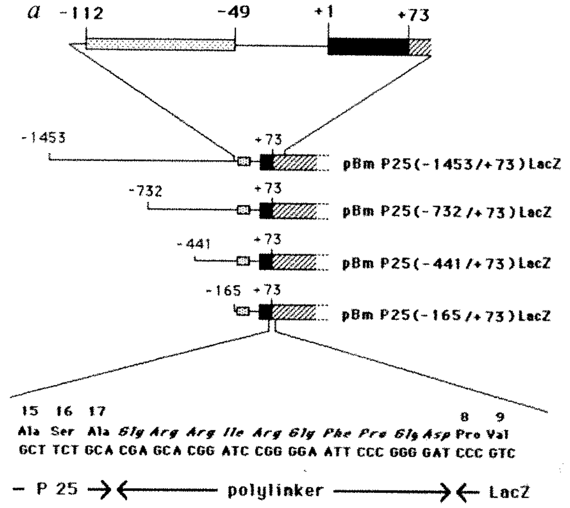
SUCCESSFUL expression of genes transferred into distantly related species in which genetic functions have been maintained through evolution has been reported previously[1–3]. In the case of the silkmoth *Bombyx mori* and the fruitfly *Drosophila melanogaster*, both of which produce chorions (eggshells), *Bombyx* chorion genes are correctly expressed in *Drosophila* despite their estimated 240-Myr phylogenetic divergence[1]. Here we report that, although *Drosophila* does not produce silk, mechanisms regulating transcription have been conserved between the salivary gland of the fruitfly and the silk gland of the silkmoth larva.

The P25 gene of *Bombyx mori* is one representative of this set of silk protein genes expressed in the posterior silk gland[4–7]. Different fusion genes were made by joining the *Escherischia coli* β-galactosidase coding sequence to the P25 gene with either 1,453, 732, 441 or 165 base pairs (bp) of P25 5′ flanking sequences. In all constructs, the fusion occurred at position +73 of the *Bombyx* gene, 54 bp downstream from the initiation codon (Fig. 1). These sequences were inserted into the vector carnegie 20, which carries the P-element terminal repeats and the gene *rosy* for phenotypic selection of transformants[8]. To account for a possible influence of the *Drosophila rosy* promoter on the


a
-112
-49
+1
+73
-1453
+73
pBm P25(-1453/+73)LacZ
-732
+73
pBm P25(-732/+73)LacZ
-441
+73
pBm P25(-441/+73)LacZ
-165
+73
pBm P25(-165/+73)LacZ
15 16 17
8 9
Ala Ser Ala Gly Arg Arg Ile Arg Gly Phe Pro Gly Asp Pro Val
GCT TCT GCA CGA GCA CGG ATC CGG GGA ATT CCC GGG GAT CCC GTC
– P 25 →← polylinker →← LacZ

b

LINE	ORIENTATION	LINKAGE	POSITION	GENOTYPE
1453 P25-Z 1	D	II	63BC	H
" " 2	D	II	84D	H
" " 3	R	II	36F	H
" " 4	R	III	ND	H
732 P25-Z 1	R	II	"	H
" " 2	R	III	"	H
" " 3	R	III	"	B
" " 4	R	III	"	B
" " 5	R	III	"	B
" " 6	R	III	"	B
441 P25-Z 1	D	III	"	H*
" " 2	D	II	"	H
" " 3	D	II	"	H
" " 4	D	II	"	H*
165 P25-Z 1	D	X	"	H
" " 2	D	III	"	B
" " 3	D	III	"	B
" " 4	D	III	"	H
" " 5	D	II	"	H
" " 6	D	II	"	H
" " 7	D	III	"	H

FIG. 1 P25–lacZ constructs used for *D. melanogaster* transformation experiments and the transformed lines analysed. *a*, Schematic representation of the chimaeric genes. P25 genomic fragments with various lengths of 5′ upstream sequences (thin line) and the first exon (filled box) were fused in frame with the *E. coli* β-galactosidase coding sequence (hatched box). Numbers represent the distances in nucleotides from the transcription start site. Dotted box, the enhancer element of the P25 gene (J. Drevet, B. Durand and P.C., unpublished results). The fused genes were inserted into *Drosophila* chromosomes using P-element mediated transformation and *rosy* selection of transformants. *b*, The transformed lines were genetically mapped and made homozygous (H) for the insert, through crosses with balancer stocks. Lethal (B) or sterile (H*) insertions were maintained over appropriate balancer chromosomes. All the lines were shown to carry a single copy of the transgene by probing Southern blots with P25 and lacZ DNA. Localization of the insert was determined by *in situ* hybridization to polytene chromosomes with a P25-Z probe. Transcriptional orientation of the fused gene was either the same (D) or the opposite (R) to that of the *rosy* gene in the transformation vector injected.

METHODS. P-element vectors containing P25–lacZ fusion genes were constructed using standard cloning procedures. pBmP25(−1,453/+73)lacZ was constructed by sequential insertion in the plasmid pUC18 of the *Hin*dIII–*Pst*I (−1,453/+73) fragment of the P25 gene and the *Pst*I–*Pst*I 3 kilobase (kb) fragment of the plasmid pMC1871 (ref. 12) containing the lacZ coding region with polylinker sequence. The same procedure was used to construct pBmP25(−732/+73)lacZ, with the *Hin*cII–*Pst*I(−732/+73) fragment. The plasmids containing P25(−441/+73) and P25(−165/+73) sequences were obtained by partial *Bal*31 digestion of pBmP25(−732/+73)DNA. End points of *Bal*31-digested DNA and the junction regions of P25 to lacZ sequences were ascertained by DNA sequencing. Fragments carrying P25–lacZ constructs were inserted into the *Hpa*I site of the carnegie 20 vector[8]. *Drosophila* transformation was as described[9] using the pπ25.7wc helper DNA. The recipient strain (*ry, st, ecdl*) was provided by J. A. Lepesant. Autosomal insertions were made homozygous by crosses with the *CyO* $TM3ry^{k}/T(2;3)Ap^{Xa}$ balancer stock. Lines that could not be made homozygous for the transgene were maintained as balanced stocks with *CYO* or *TM3* chromosomes. The X-linked insertion was made homozygous by inbreeding.

expression of the fused gene, their respective transcriptional orientation within the vector was made either the same or opposite (Fig. 1). The constructs were injected with helper DNA (pπ25.7 wc) (ref.9) into *Drosophila rosy* and *scarlet* embryos. Transformant lines were made homozygous or were stabilized with appropriate balancer chromosomes in the case of lethal insertions. Four to seven independent lines were established per construct, each being characterized by a unique *Drosophila* genomic DNA insertion site (Fig. 1).

The expression of the transgene was assayed by histological detection of the bacterial β-galactosidase activity using X-Gal (5-bromo-4-chloro-3indolyl-βD-galactopyranoside) as chromogenic substrate on tissues of animals taken at different stages, from early first larval instar to pigmented puparium, and during early and late adult life. The staining pattern of each line was compared to that of the recipient strain and to that of other laboratory stocks.

In all the lines transformed with either the 1,453, the 732 or the 441 P25-Z fusion genes and whatever their orientation within the vector, expression was detected in the larval—but not in the adult—salivary glands, where no endogenous activity was found in controls. No other organs displayed detectable staining, except the midgut, where staining developed as in the control strains. To confirm that the salivary gland staining was dependent on the expression of the transgene, we detected the enzyme in this tissue on polyacrylamide gels. The active protein migrated with a relative molecular mass (M_r) of ~520,000 (520K), a little slower than the pure homotetramer of the *E. coli* enzyme—consistent with the presence of 20 extra amino acids in the fusion protein—identifying it as the product of the transgene. As expected, the *Drosophila* endogenous enzyme migrated with an apparent M_r of 160K (ref. 10) (gel not shown).

It is of interest that the activity of the transgene is restricted to the anterior salivary glands of *Drosophila*, whatever the larval stage or the construct analysed (Fig. 2). The sharp transitions between the β-galactosidase-positive and -negative cells delineate a group of 16-24 cells in the most anterior part of the gland proper and immediately adjacent to the duct cells. To our knowledge, the functional specialization of this territory with a discrete number of cells had not been described before[11] and remarkably, it occurs at an early stage of larval life.

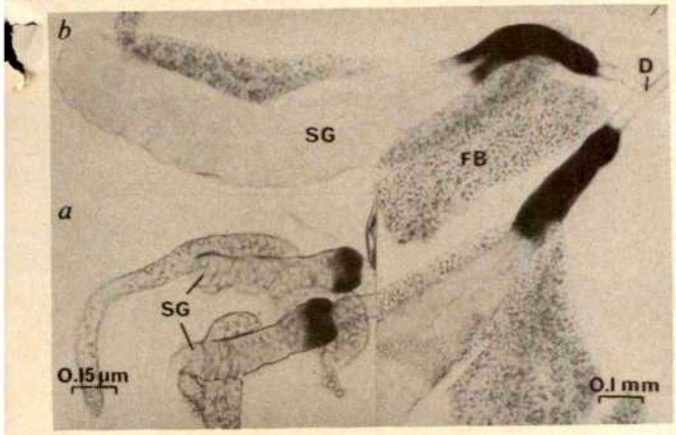


FIG. 2 Typical β-galactosidase activity in anterior salivary glands of transformed *Drosophila*. Pictures show X-Gal staining of whole glands from larvae of the line 1,453 P25-Z 3 taken at the first larval instar (*a*) and at mid-third larval instar (*b*). β-Galactosidase histochemical detection was on whole dissected animals[13]. Transgene-dependent blue staining was observed only in the larval anterior salivary glands in the lines transformed with constructs carrying 1,453, 732 or 441 bp of P25 5′ flanking DNA. Colour developed within 30 min–4 h in these lines, whereas no staining was detected until at least 48 h in the lines carrying 165 bp of 5′ flanking sequence or in the controls (not shown). SG, salivary gland; D, duct; FB, fat body lobe.

Lack of β-galactosidase expression in the 165-P25-Z transformants limits the *cis*-elements needed for expression in *Drosophila* anterior salivary glands cells to within −441/−165, a region upstream of the enhancer of the gene (Fig. 1).

To characterize the level of expression of the transgene more thoroughly, we quantified the β-galactosidase activity in the

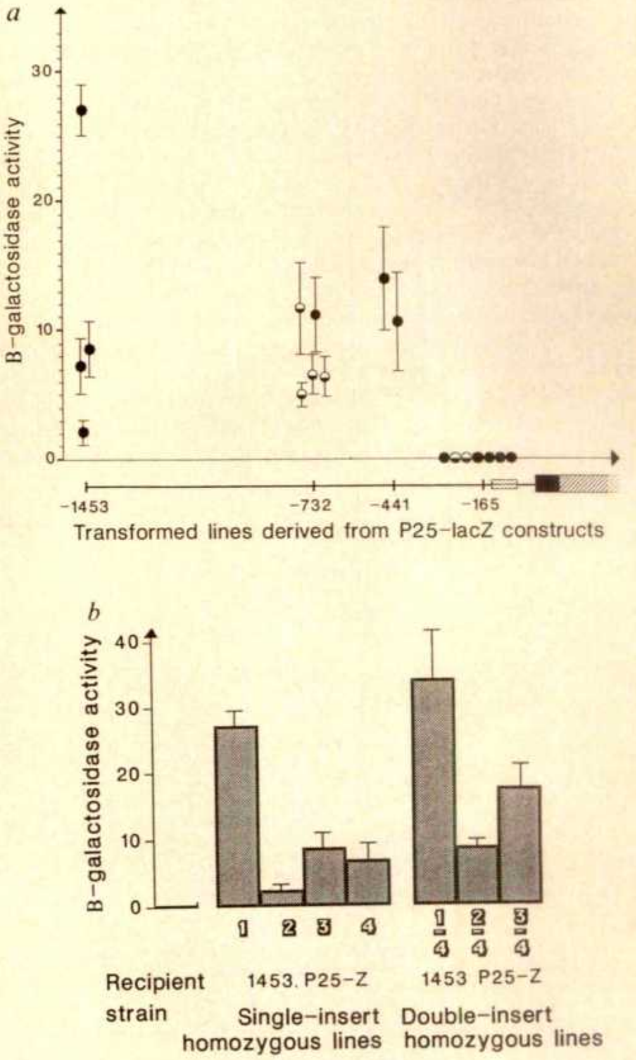


FIG. 3 Quantitative analysis of β-galactosidase activity in transformed strains carrying the P25-lacZ fusion gene and deletion mutants. *a*, Enzyme activity of salivary gland extracts prepared from wandering third instar larvae. For each transformed line, data were obtained from at least 3 independent sets of glands. The P25 flanking DNA is depicted at the bottom of the figure as in Fig. 1. Lines 441 P25-Z 1 and 2, having a very low fertility, were not included in the quantitative analysis but revealed expression of the transgene by histochemical tests. (●, Homozygous lines; ◒, heterozygous balanced lines. *b*, Effects of gene dose on β-galactosidase activity. Double-insert lines carrying the 1,453 P25-Z fusion gene were obtained by crossing line 4 (insert on the third chromosome) to each of the lines 1, 2 and 3 (insert on the second chromosome). The double homozygous lines were assayed for β-galactosidase activity as in *a*, except that 8–13 independent assays were performed per line. β-galactosidase activity is given as absorbance$_{574}$ $\times 10^{-3}$ h^{-1} per 10 pairs of salivary glands.

METHODS. β-galactosidase activity was determined spectrophotometrically by following the hydrolysis of chlorophenol-red β-0-galactopyranoside (CRPG)[14]. Assay conditions were as follows: 10 pairs of salivary glands dissected from third instar wandering larvae were homogenized in 1 ml of 1mM $MgCl_2$ and 50 mM potassium phosphate, pH 7.5. Debris were removed by centrifugation and the supernatant was made 1 mM in CRPG. Reaction mixtures were incubated at 37 °C and the absorbance at 574 nm was monitored during the first 6 h, during which the rate of colour development was constant.

salivary glands of the different lines. As shown in Fig. 3, there is a drastic loss of activity between the transgenic lines 1,453-, 732- and 441-P25-Z and the lines 165-P25-Z, in agreement with the results of the histochemical tests. We interpret the differences between the lines as being due to position effects. Also, we found that lines containing double inserts show a level of β-galactosidase activity that corresponds to the sum of each of the two single insert lines (Fig. 3*b*). We conclude that there is no limitation on transactivating properties in the transformed lines, and that the level of expression of the transgene depends only on its chromosomal position.

Here we show that although no silk production occurs in *Drosophila*, its salivary glands contain *trans*-acting factors capable of recognizing *cis* elements of a *Bombyx* silk protein gene, and of conferring positional and temporal characteristics on its expression. Thus, although the functions of salivary and silk gland cells have diverged, they probably involve evolutionarily conserved regulatory signals. Our observation that only cells of the anterior region of the salivary gland express the transgene suggests that they are homologous to the cells of the posterior silk gland. To further elucidate conservation of transcriptional regulatory mechanisms, we are determining which of the *cis*-acting elements of the P25 promoter are recognized in *Drosophila* and testing whether genes encoding the silk proteins synthesized in the middle silk gland are also expressed in *Drosophila* salivary glands. □

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Mediation of *Drosophila* head development by gap-like segmentation genes

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THE first phase of embryonic development in *Drosophila* consists of the elaboration and interpretation of maternally encoded information that specifies spatial pattern in the embryo[1]. The product of the maternal gene *bicoid (bcd)* is thought to organize the anterior pattern of the embryo[2]. Although the *bcd* transcript is localized at the anterior pole of the egg[3,4] the *bcd* protein forms a stable concentration gradient through the anterior two thirds of the embryo[5]. The graded distribution of *bcd* protein defines position along the anterior–posterior axis of the embryo[6–8] through the spatially restricted activation of subordinate targets such as the gap gene *hunchback (hb)*[7–11]. *In vitro* manipulation of specific *bcd* protein binding sites has shown that the gradient of *bcd* protein can in principle define more than one discrete domain of spatially restricted gene activation in the head of the embryo, depending on the affinity of the available binding sites for the *bcd* protein[8,12]. Genetic analysis has indicated the need for at least one additional zygotic segmentation gene to mediate *bcd* function in portions of the head that lie anterior to the *hb* domain[2,12–14]. The missing gene activity is expected to be activated in response to higher levels of *bcd* protein than are required for *hb* activation[12]. We report here that three previously identified zygotic genes *buttonhead* (*btd*), *empty spiracles* (*ems*) and *orthodenticle* (*otd*)[15–17] may behave like gap genes that mediate *bcd* function in the embryonic head.

Our analysis of *btd*, *ems* and *otd* was initiated in the search for genes that control expression of the *Distal-less* (*Dll*) gene in the head segments of the embryo. *Dll* function is required for limb development in the embryo[18,19] and the *Dll* transcript is expressed in the primordia of the limbs from early stages of development[20]. Previous analysis indicated that *Dll* expression in the thoracic and gnathal segments of the embryo depends on the segment-polarity genes *engrailed* (*en*) and *wingless* (*wg*), but activation of *Dll* expression in the antennal segment does not[20]. We monitored *Dll* expression in embryos mutant for genes in which the antennal sense organ fails to develop, but in which other *Dll*-dependent structures, like the maxillary sense organ, are normal. In embryos mutant for three such genes—*btd*, *ems* and *otd*—*Dll* is not expressed in the primordium of the antenna, but is expressed in the primordia of the other limbs (Fig. 1 *a*–*d*). No defect in *Dll* expression was observed in embryos mutant for a fourth gene, identified by a single allele called XQ (ref. 15; data not shown).

Careful examination of the cuticular phenotypes of *btd*, *ems* and *otd* mutants indicated that these genes are required in overlapping but non-identical domains in the embryonic head (Fig. 2). This analysis suggests that *btd* activity is required for development of mandibular, intercalary and antennal structures (Fig. 2, *d*–*f*). The activity of *ems* is required for development of intercalary and antennal structures and portions of the head skeleton from the pre-antennal region[17] (Fig. 2*g*–*i*). Some mandibular structures lacking in *btd* mutants are present in *ems* mutants, suggesting that although they overlap to some extent, *ems* acts more anteriorly. Similarly, *otd* mutant embryos lack antennal and pre-antennal structures (Fig. 2 *j*–*l*). Embryos mutant for *otd* develop structures from the intercalary segment that are defective in *ems* mutants and lack more of the pre-antennal head structures than do *ems* mutant embryos, suggesting that *otd* is required in a more anterior domain than is *ems*.

As structural defects in the head skeleton are often difficult to interpret and not all structures can be scored unambiguously (see legend to Fig. 2), we monitored the effects of the mutants on the expression of *en* and *wg*, which label individual segments of the embryonic head in very characteristic patterns[21,22] (Fig. 1, *e*, *i*). Consistent with the results of the cuticular analysis, the mandibular, intercalary and antennal segments are absent in the *btd* mutant embryo. Similarly, the intercalary and antennal segments and a portion of the pre-antennal region defined by the *en* head spot are deleted in *ems* mutant embryos. The antennal segment and a greater portion of the pre-antennal region, including the region expressing *wg*, are deleted in *otd* mutant embryos. Mutations in each of the genes cause the deletion of adjacent segments in the head (presented schematically in Fig. 3). The domains in which *btd*, *ems* and *otd* act, overlap in the antennal segment but each is out of phase with its neighbour(s) by one segment. There is no indication of homeotic transformation of the identities of the affected segments, rather these segments are absent. Therefore *btd*, *ems* and *otd* seem to be required to establish contiguous blocks of segments. In this respect their function is similar to that of the gap genes like *hb*, *Krüppel* and *knirps* (*kni*), which specify overlapping domains in the embryonic trunk.

If the *btd*, *ems* and *otd* genes serve as zygotic interpreters of the maternal positional information in the head, we expect that their activity should be required during early embryogenesis, as for the gap genes. The phenotypic criteria that suggest a gap gene-like role for *btd*, *ems* and *otd* are based on morphological and molecular markers expressed at a relatively late stage of development. We therefore sought indications that these genes might function early. The *btd* gene can be shown to function at the blastoderm stage in two independent ways. First, the pattern of *Dll* expression is altered in *btd* mutant embryos during cellularization of the blastoderm (Fig. 4*a*, *b*). Second, the activity of *btd* is required for the activation of the anterior ring of expression of the gap gene *kni* at the cellular blastoderm stage (data not shown). This *kni* stripe lies just anterior to the presumptive cephalic furrow, in what will be the mandibular segment. The activity of *ems* is not required for activation of the *kni* stripe, even though most of the cells in the *kni* stripe also express *ems* (data not shown). These observations are consistent with the suggestions, based on the later phenotypes, that *btd* is required for development of the presumptive mandibular segment and that the posterior limit of the domain of *ems* action is anterior to that of *btd*. Although the pattern of *btd* expression is not known, the domain in which it appears to be required at the blastoderm stage is consistent with the later requirement in the mandibular, intercalary and antennal segments.

Both *otd* and *ems* exhibit many of the features that would be expected of genes that fulfil the necessary *bcd*-dependent, gap gene-like function in the head: *ems* is first expressed during cellularization of the blastoderm in a single stripe in the head that corresponds to the locations of the primordia of the structures affected in mutant embryos[17]; the *otd* gene product is expressed in a somewhat broader stripe in the head during cellularization[32]. Expression of both genes depends on *bcd* and the position at which their respective stripes are expressed along the anterior-posterior axis is dependent on *bcd* copy number

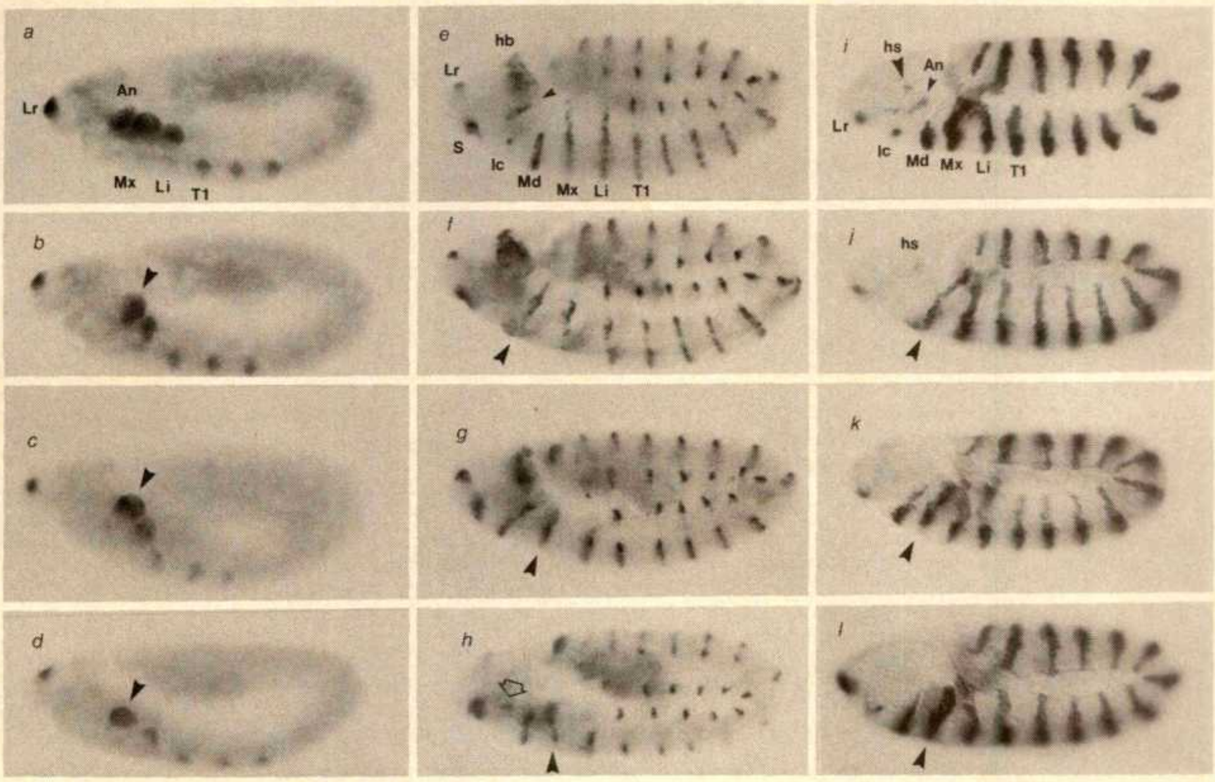

FIG. 1 Embryos mutant for *btd*, *ems* and *otd* exhibit phenotypes characteristic of gap genes. *a*–*d*, *Distal-less* expression in wild-type, *btd*, *ems* and *otd* embryos, respectively. Embryos mutant for the three genes lack *Dll* expression in the primordium of the antenna. Arrowhead indicates the maxillary limb primordium. For a detailed description of the *Dll* expression pattern see ref. 20. *e*–*h*, *wingless* and *i*–*l engrailed* expression in wild type, *btd*, *ems* and *otd* embryos, respectively. The patterns of *wg* and *en* expression are very characteristic in the head segments[21,22]. Each segment can be clearly identified, as indicated in *e* and *i*. Embryos mutant for *btd* lack expression of both *wg* and *en* in the mandibular, intercalary and antennal segments. The maxillary segment (large arrowhead) is unaffected. The pre-antennal region of the head is not affected, as monitored by the pre-antennal head spot of *en* (hs) and the head blob of *wg* expression (hb). The deletion seems to be in segmental, rather than parasegmental, register as *en* and *wg* stripes are always affected together in a given segment. Embryos mutant for *ems* lack expression of both *wg* and *en* in the intercalary and antennal segments and in a portion of the pre-antennal region of the head. Note that the pre-antennal head spot of *en* is missing, but that the *wg* head blob is still present. Expression of both genes is present in the mandibular segment. Embryos mutant for *otd* lack expression of both *wg* and *en* in the antennal segment and in the pre-antennal regions of the head defined by both *en* and *wg* expression. Both genes are expressed in the mandibular and intercalary segments. Open arrow in *h*, intercalary expression of *wg*. Abbreviations; T1, first thoracic; Li, labial; Mx, maxillary; Md, mandibular; Ic, intercalary; An, antennal segments; hs, the head spot of *en* expression; hb, the more anteriorly located head blob of *wg* expression; Lr, labrum; S, stomodeum. The small arrowhead in *e* indicates the antennal segment.

METHODS. *Dll* and *wg* expression were visualized by whole-mount *in situ* hybridization[28], modified as described[20]. Expression of *en* was detected by labelling with a monoclonal antibody (kindly provided by P. A. Lawrence) as described[29]. The *btd* gene is defined by 3 mutants (designated XO, XA, XG and is included in a deficiency Df(1) C52, see ref. 16). As no duplication exists that covers the locus, these lesions have not been directly tested for allelism. All of the putative alleles including the Df, map genetically (to position 31) into the small interval between *almondex-lozenge* and *vermillion* (map positions 27.7 and 33.0 respectively). XO and XG produce a strong phenotype, indistinguishable from the Df. XA produces a weaker phenotype in which mandibular segment is often not affected. The cytogenetic location of *btd* is delimited to the region 8E4-9A1 by Df(1) C52 (8E4; 9C) and the duplication Dp(1; 2) v^{+75d} (9A2-10C; 40-41), which does not include *btd*. The *ems* alleles 9H and 9Q encode non-functional proteins in which termination codons occur before the homeodomain and therefore show the complete lack of function phenotype[17]. Both a deletion (Df(1) KA14) and a point mutant allele (YM) of *otd* (ref. 15) exhibit the same phenotype.

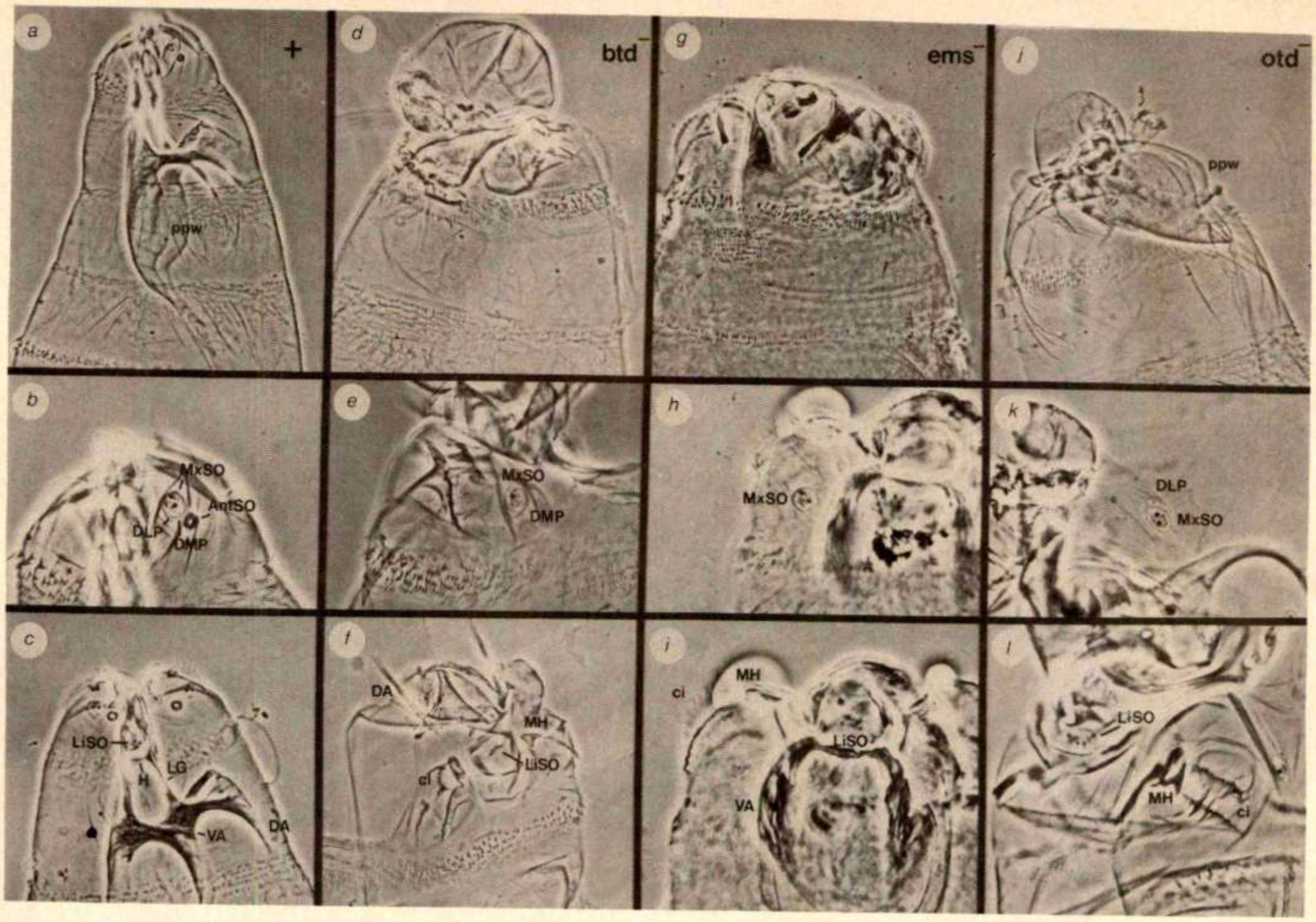

FIG. 2 The genes *btd*, *ems* and *otd* affect overlapping but distinct regions of the head. Cuticular phenotypes of the head region of wild-type (*a*—*c*), *btd* (*d*–*f*), *ems* (*g*–*i*) and *otd* (*j*–*l*) embryos. The segmental origin of the structures of the embryonic head have been determined by ultraviolet-laser-ablation fate mapping[30]. Structures derived from the labial and maxillary segments are present in all the mutants. Labial sense organ (LiSO), cirri (ci), mouth hooks (MH), Maxillary sense organ (MxSO). The labrum is also present in all mutants, but is difficult to recognize in embryos where head involution has failed, as are other elements of the head skeleton from the labial segment. Embryos mutant for *btd* lack the ventral arms (VA), of mandibular origin. The Intercalary segment contributes the posterior wall of the pharynx (ppw), which is reduced in *btd* (not shown). The antennal sense organ (AntSO) is missing. The dorsal arms (DA) of the head skeleton, which derive from the pre-antennal region, are present. The dorso-medial papilla is present (DMP). This structure was originally described as being of antennal origin, as fate mapping studies place it close to the antenna. The present data suggest that the DMP may be of pre-antennal origin, possibly deriving from a position close to the *en* head spot as the DMP is missing in *ems* mutant embryos. Similarly the dorso-lateral papilla (DLP) is present in *otd* but absent in *ems*, which suggests that the DLP might be intercalary in origin, rather than mandibular as proposed previously[30]. These ambiguities in the previously proposed segmental origins lie well within the experimental uncertainty of the fate mapping experiments, as all of the structures map very close together near the boundary region between the head segments (see Fig. 8 in ref. 30). Mandibular structures like the VA are present in *ems* and *otd*, but such structures are difficult to identify clearly in *otd* because of the more extreme defects in head involution. Note that intercalary structures such as the ppw develop normally in *otd* mutant embryos (compare *a* and *j*).

(ref. 17, and R. Finkelstein *et al.*, personal communication). The suggestion that *btd*, *ems* and *otd* might be direct targets of *bcd* protein is strengthened by the observation that *bcd* protein expressed in salivary glands binds to the cytogenetic loci where the *ems* and *otd* genes are located (W. Driever and C. Nüsslein-Volhard, personal communication). It remains to be determined whether *bcd* directly activates any of these genes in a concentration-dependent manner. The fact that both *ems* and *otd* encode homeobox-containing proteins[17,32] is consistent with their proposed function as gap-like segmentation genes required to promote the development of head segments.

Several lines of evidence suggest that the process of subdivision of the head into metameric units may differ fundamentally from that of the trunk. In the trunk, combinatorial inputs from the gap genes establish the domains of pair-rule gene expression[23,24], which in turn define the metameric patterns of segment-polarity gene expression[25] (reviewed in refs 26, 27). In the head, *btd*, *ems* and *otd* seem to function like gap genes as they are required to specify broad overlapping domains. However, the segmentally repeated patterns of *en* and *wg* are established in this region without input from pair-rule genes. One scenario that might explain the generation of a segmented pattern in the head is based on the observation that the posterior limits of the domains of action of *btd*, *ems* and *otd* are out of phase by one segment. It is possible that segmentation of the head is specified directly by a combinatorial input of information from these—and possibly other—as-yet-unidentified genes. This suggestion derives in part from the observation that although the *btd*, *ems* and *otd* domains overlap considerably, we were unable to demonstrate any hierarchical relationship among them. The expression of *ems* is not obviously affected in either of the other two mutants (data not shown). If *btd*, *ems* and *otd* are direct mediators of the anterior positional information encoded by the maternal *bcd* protein gradient, they might accomplish simultaneously what the gap and homeotic selector genes do in the trunk of the embryo. □

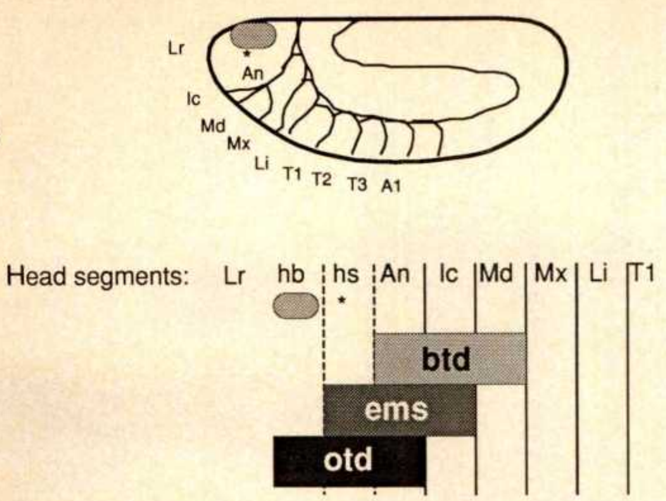

FIG. 3 Schematic representation of the segmental defects in the mutants. The *btd* gene is required for development of the mandibular, intercalary and antennal segments, but not for maxillary and pre-antennal regions. The *ems* gene is required in intercalary and antennal segments and in a part of the pre-antennal domain—defined by the *en* head spot (hs)—but not in more anterior pre-antennal regions—defined by the *wg* head blob (hb)—or in the mandibular segment. The *otd* gene is required in the antennal segment and both pre-antennal regions, but not in intercalary or mandibular segments. The domains in which the genes are required overlap and are out of phase by one segment at their posterior limits. The anterior limit of the domain of action of *btd*, *ems* and *otd* are also out of phase, occupying progressively greater portions of the pre-antennal region. Their effects on *en* and *wg* expression anteriorly may reflect cryptic segmentation in the pre-antennal region of the head. These domains (hs and hb) are indicated with dashed lines to point out that they may not be true segments. Jürgens *et al.*[30] and Struhl[31] both propose six head segments. None of the genes analysed here affects the labral segment (Lr).

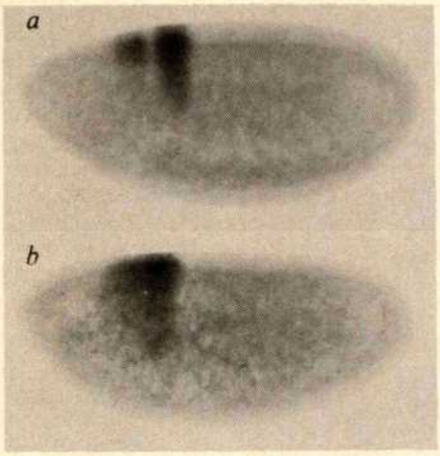

FIG. 4 The pattern of expression of *Dll* is altered at the blastoderm stage in *btd* mutant embryos. *Dll* is first expressed during cellularization of the blastoderm, in two bands that are restricted in their dorsal-ventral extents[20]. Double-labelling with probes that detect *Dll* and *wingless* shows that the more posterior band corresponds to the primordium of the maxillary and labial limbs (which resolves at a later stage into discrete primordia; data not shown). Following the development of the mature pattern of *Dll* expression suggests that the anterior band corresponds to the presumptive antennal primordium[20]. In *btd* mutant embryos only one broadened stripe of *Dll* expression is seen at the blastoderm stage. As the mandibular, intercalary and antennal segments are absent at later stages in *btd* mutant embryos, it seems likely that the broadened *Dll* band observed reflects a deletion of the early antennal primordium with a concomitant anterior expansion of the maxillary-labial stripe. In the absence of hybridization probes that distinguish these primordia at the blastoderm stage, this suggestion cannot be directly tested.

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The *orthodenticle* gene is regulated by *bicoid* and *torso* and specifies *Drosophila* head development

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IN the *Drosophila* embryo, cell fate along the anterior–posterior axis is determined by maternally expressed genes[1,2]. The activity of the *bicoid* (*bcd*) gene is required for the development of larval head and thoracic structures[3], and that of maternal *torso* (*tor*)[1] for the development of the unsegmented region of the head (acron). In contrast to the case of thoracic and abdominal segmentation[4,5], the hierarchy of zygotically expressed genes controlling head development has not been clearly defined. The *bcd* protein, which is expressed in a gradient[6], activates zygotic expression of the gap gene *hunchback* (*hb*)[7,8], but *hb* alone is not sufficient to specify head development. Driever *et al.*[9] proposed that at least one other *bcd*-activated gene controls the development of head regions anterior to the *hb* domain. We report here that the homeobox gene *orthodenticle* (*otd*), which is involved in head development, could be such a gene. We also show that *otd* expression responds to the activity of the maternal *tor* gene at the anterior pole of the embryo.

Mutations at the *otd* locus cause embryonic lethality, head involution fails to occur properly and several larval cuticular and sensory head structures are deleted or grossly perturbed[10]. The deleted elements are antennal or pre-antennal in their segmental origin (Fig. 1). Structures corresponding to the labral, intercalary and gnathal (mandibular, maxillary, and labial) segments develop, but are often disrupted, probably as a result of abnormal head involution. No evidence for homeotic transformation or duplication of head structures is found in *otd* embryos. Analysis of the cuticular phenotype of the head, which

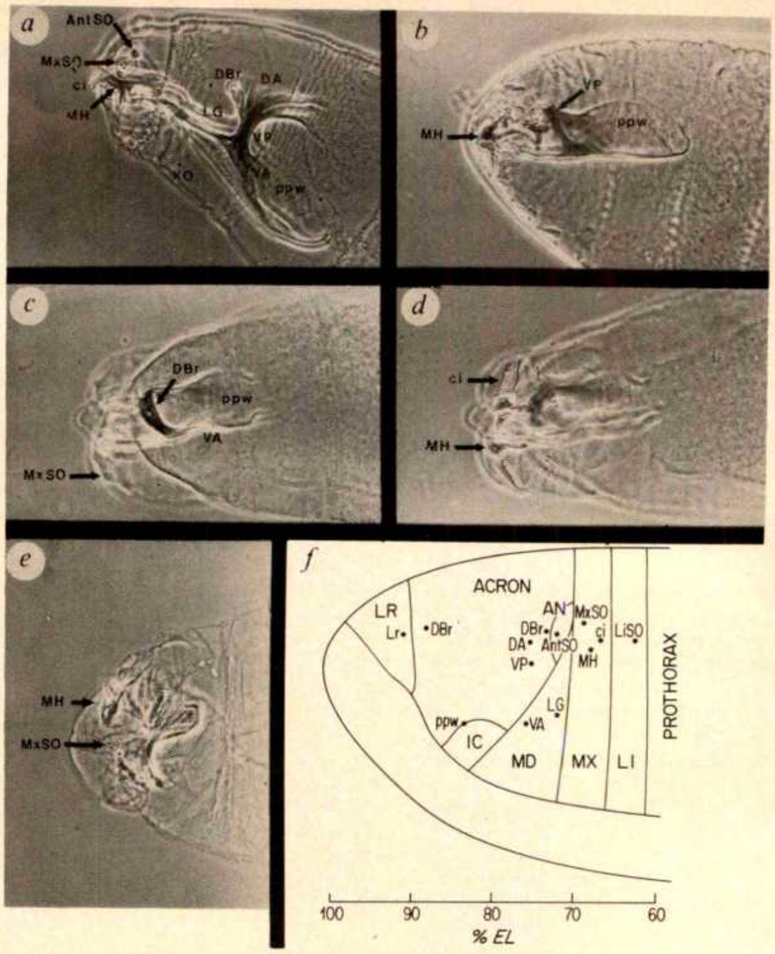


FIG. 1 Head defects in *otd* embryos. *a–e*, Phase contrast micrographs of cuticular preparations of wild-type (*a*) and *otd* mutant embryos (*b–e*). For abbreviations, see below. For a detailed description of wild-type head cuticular structures and sense organs, see ref. 24. Anterior is to the left in all panels. *b* and *c*, VA and ppw, which are of mandibular and intercalary origin respectively. The DBr and VP, additional elements of the cephalopharyngeal skeleton, are also present. The DA, which are derived from the pre-antennal region, are either absent or fragmented. *d*, Derivatives of the maxillary segment, the cirri and mouth hooks, are present in *otd* embryos. *e*, Absence of the AntSO and the presence of the MxSO; the LiSO is also present (not shown). The dorsal–medial papilla, which is probably pre-antennal in origin (S. Cohen and G. Jurgens, personal communication) is deleted (not shown). *f*, Fate map of the embryonic head at the blastoderm stage (adapted from Jurgens *et al.*[24]). Segmental assignment is based on the defects produced by laser irradiation at this stage of development. The labral, antennal, intercalary, mandibular, maxillary and labial segment boundaries are demarcated, and the fate map positions of several head structures indicated. Abbreviations: antennal sense organ, AntSO; antennal segment, AN; cirri, ci; dorsal arms, DA; dorsal bridge, DBr; egg length, EL; intercalary segment, IC; Keilin's organ, KO; labial sense organ, LiSO; labial segment, LI; labral segment, LR; labrum, lr; lateralgrate, LG; mandibular segment, MD; maxillary segment, MX; maxillary sense organ, MxSO; mouth hooks, MH; posterior wall of the pharynx, ppw; ventral arms, VA; vertical plate, VP.

METHODS. Cuticles were prepared as described previously[25]. The cuticular phenotypes shown are similar for two ethylmethane sulphonate-induced mutant alleles, otd^{XD87} and otd^{YH13}, and one deficiency allele, otd^{JA101} (refs 10, 30).

is difficult in first instar larvae, is supported by the expression patterns of the segment polarity genes *engrailed* (*en*) and *wingless* (*wg*) in the developing embryo (Fig. 2*a*, *c*). In addition to the reiterated stripes marking the gnathal, thoracic and abdominal segments, each of these genes is expressed in a specific, segmental pattern in more anterior head regions (refs 11-13; S. Cohen, personal communication). In *otd* mutant embryos, the expression of *en* and *wg* in antennal and pre-antennal regions is deleted (Fig. 2*b*, *d*). Consistent with the cuticular *otd* phenotype, expression corresponding to the labral, intercalary and gnathal segments is present in *otd* embryos.

To determine the relationship between the head phenotype observed in mutant embryos and *otd* expression, we analysed the early pattern of *otd* transcription (Fig. 3). Transcription is evident before cellularization at stage 4 of embryonic development and is restricted to a circumferential anterior region extending to the pole of the embryo. By the time cellularization is complete, *otd* expression has retracted from the pole and is confined to a broad circumferential stripe extending from 70 to 90% of the egg length, which subsequently diminishes in intensity ventrally. Expression is also detectable in the yolk nuclei within the region of the stripe (data not shown). The

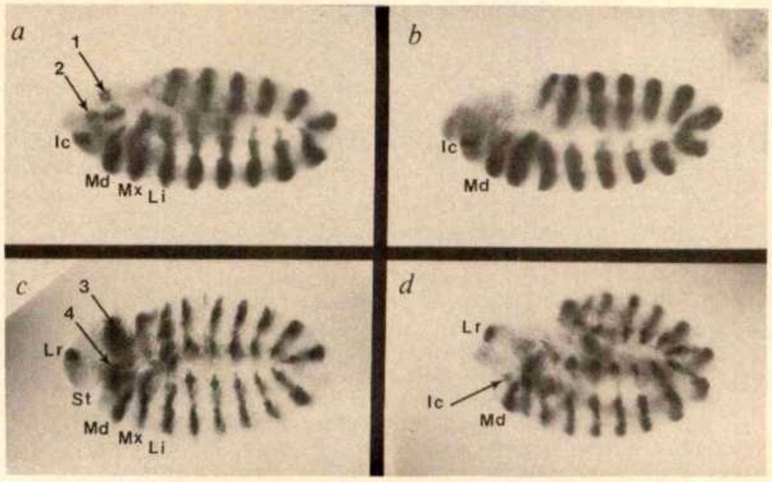
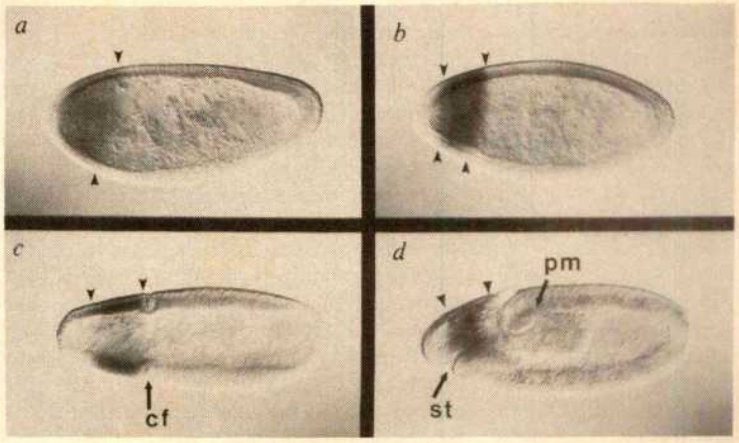


FIG. 2 Specific elements of *engrailed* (*en*) and *wingless* (*wg*) head expression are deleted in *otd* mutant embryos. *a* and *b*, Expression pattern of *en* at the germ band-extended stage in wild-type and *otd* embryos, respectively. In wild-type embryos, *en* is expressed anterior to the gnathal segments (Md, Mx and Li) in the intercalary (Ic) and antennal (arrow 2) segments (ref. 11; S. Cohen, personal communication). In addition, a pre-antennal spot of expression (arrow 1) is visible. In *otd* embryos, the antennal and pre-antennal regions of *en* expression are deleted, but expression in the intercalary and gnathal segments is retained. *c* and *d*, Expression pattern of *wg* in wild-type[12,13] and *otd* embryos, respectively. Expression in the stomodeal (St), labral (Lr), antennal (arrow 4) and pre-antennal (arrow 3) regions is visible. Expression in the intercalary segment is present, but cannot be seen in this focal plane. In an *otd* embryo (*d*), the deletion of the regions of antennal and pre-antennal staining can be seen. Expression of *wg* marking the Ic and Lr segments is retained. In all panels, anterior is to the left and dorsal at the top.

METHODS. Expression of *en* and *wg* were monitored using two *lacZ* insertion strains that accurately reproduce the embryonic patterns of protein expression of *en* (C. Hama and T. Kornberg, personal communication; ref. 26) and *wg* (N.P. and J. Kassis, unpublished observations). Females heterozygous for *otd* mutations were crossed with males from either *lacZ* strain. The *lacZ* expression was detected using an antibody to β-galactosidase with processing as previously described[26].

FIG. 3 Early expression of *otd* during embryogenesis. In all panels, anterior is to the left and dorsal at the top, except for *c* which is a dorsal view. Small arrows indicate the anterior–posterior boundaries of the domains of *otd* transcription. *a*, Expression of *otd* in a late stage-4 embryo; transcription can be seen in the anterior region. *b*, Cellularized stage-5 embryo; *otd* expression has retracted from the anterior pole, and forms a circumferential stripe extending from 70 to 90% egg length. Transcription diminishes in the ventral region of the stripe during subsequent stages. *c*, During early gastrulation in stage-6 embryos, the cephalic furrow forms just posterior to the domain of *otd* transcription. In germ band-extended embryos (stage 8), *otd* expression is localized to the procephalic head region. Staging of embryos was according to Campos-Ortega and Hartenstein[27]. Abbreviations: cephalic furrow, cf; stomodeum, st; posterior midgut, pm.

METHODS. *In situ* hybridization to whole-mount embryos was performed according to Tautz and Pfeifle[28]. The probe used for digoxygenin-labelling was a 3.8-kilobase *otd* complementary DNA (ref. 30).



formation of the cephalic furrow occurs just posterior to the domain of *otd* expression. By the extended germ-band stage, *otd* transcription is localized within the head region.

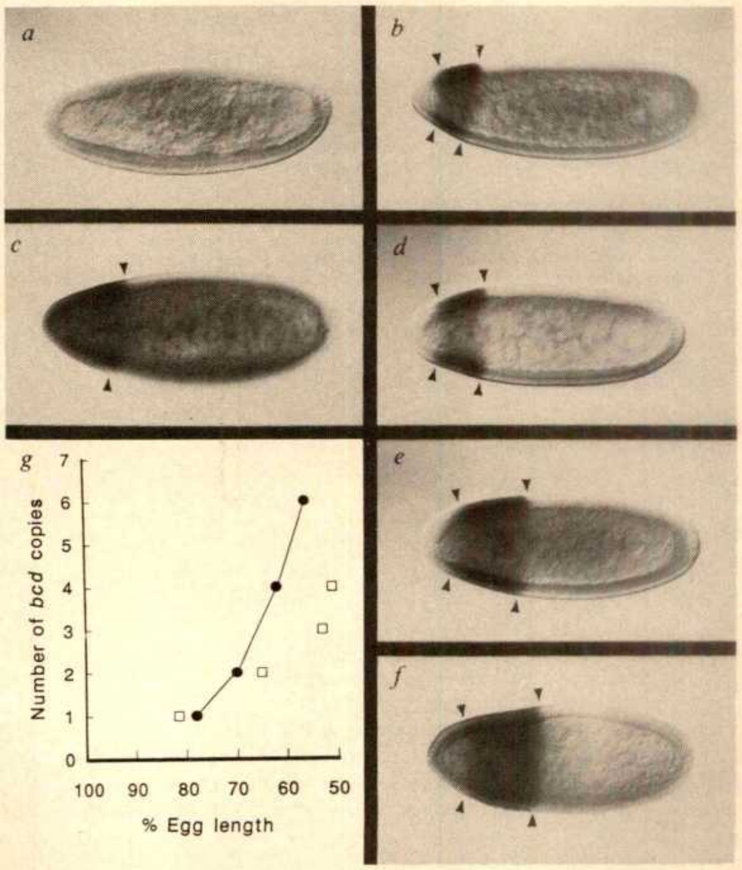
We have previously characterized the *otd* gene and shown that it encodes a predicted homeodomain protein[30]. Homeodomains can be classified according to the amino acid at position 9 of the putative recognition helix, which has been shown to confer binding specificity[14,15]. The *otd* and *bcd* gene products[16,17] are the only known proteins with a lysine residue at this position of the homeodomain.

The expression of *otd* is positively regulated by *bcd* in a concentration-dependent fashion (Fig. 4*a–g*). In embryos lacking maternal *bcd* product, *otd* transcription is absent. As the number of copies of *bcd* increases, the domain of *otd* transcription extends progressively towards the posterior end of the embryo. The expression of *otd* is negatively regulated by *tor*; in the absence of maternal *tor* activity, *otd* transcription fails to retract from the anterior 10% of the embryo (Fig. 4*c*). A similar effect has been observed for zygotic *hb* expression[9].

As the effect of *hb* mutations on head development[18] does not account for the absence of all head structures in *bcd* embryos the existence of an additional *bcd*-regulated gene(s) has been proposed[9]. Driever *et al.*[9] postulated the existence of a gene(s) which would be activated by higher *bcd* concentrations than is *hb* and specify all or part of the anterior head region. The predicted domain of expression of such a gene would therefore be localized within the anterior region of the *hb* domain of transcription. The *otd* gene, which is required for the development of structures from the antennal and pre-antennal head segments, fulfills these criteria. In addition to *otd*, the gene *empty spiracles* (*ems*)[19], which is also required for head development, is expressed in a circumferential stripe in the anterior region of

FIG. 4 Expression of *otd* is regulated positively by *bcd* and negatively by *tor*. *a–f*, *In situ* hybridization to embryos of various maternal genotypes. Anterior, left; all views are dorsal except *b*, which is lateral. *a*, *b*, *d*, *e* and *f*, Embryos derived from mothers carrying 0, 1, 2, 4, and 6 copies, respectively, of the wild-type *bcd* gene. The posterior extent of *otd* transcription is progressively extended as *bcd* dosage increases; *g* depicts this shift graphically. The *otd* points (●) indicate the position of the posterior limit of *otd* expression as a function of maternal *bcd* dosage. In all cases, measurements were made using dorsal views. In addition, the effect of maternal *bcd* dosage on *bcd* protein expression is shown. The *bcd* points (☐) indicate the egg length position at which the same level of *bcd* protein is found for embryos derived from females with varying *bcd* dosages. The 65% value of *bcd* immunostaining intensity was chosen arbitrarily. Data is derived from ref. 29. *c*, Expression of *otd* in an embryo lacking maternal *tor* product; *otd* expression fails to retract from the anterior pole at the cellular blastoderm stage. Arrowheads in all panels show the anterior and posterior position of the domain of *otd* expression.

METHODS. *In situ* hybridization was as in Fig. 3. The genotypes of the females that produced embryos with 0, 1, 2, 4 and 6 copies of bcd^+ were respectively: bcd^{E1}/bcd^{E1}, $bcd^{E1}/\pm$, ±/±, *BB9* + *BB16*/± and *BB9* + *BB16*/*BB9* + *BB16*. The *BB9* + *BB16* chromosome contains three copies of a wild-type *bcd* gene (G. Struhl, personal communication). The *tor* mutant embryos were derived from tor^{wk34}/tor^{wk34} homozygous females[1].



the embryo[20]. Both the *otd* and *ems* genes encode predicted homeodomain proteins and are dependent on the level of maternal *bcd* gene product for the establishment of their domains of expression. Mutations at each of these loci cause the deletion of subsets of adjacent head segments and show no evidence for the homeotic transformation of structural elements. If these genes are indeed directly transcriptionally activated by *bcd* (as suggested by the early appearance of their expression), then the regulation of *Drosophila* embryonic head development may be different from that of the thoracic and abdominal regions. In those regions, the earliest zygotic regulation is provided by the gap genes, several of which encode zinc-finger-containing proteins[21-23] required for the sequential activation of the downstream pair-rule and segment polarity genes. In the head, however, homeobox genes such as *otd* and *ems* may function as direct intermediaries between the *bcd* morphogen and the segment polarity genes. □

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Detection and characterization of a folding intermediate in barnase by NMR

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PROTEIN engineering is being developed for mapping the energetics and pathway of protein folding. From kinetic studies on wild-type and mutant proteins, the sequence and energetics of formation of tertiary interactions of side chains can be mapped and the formation of secondary structure inferred[1,2]. Here we cross-check and complement results from this approach by using a recently developed procedure that traps and characterizes secondary structure in intermediate states using ^{1}H NMR[3,4]. The refolding of barnase is shown to be a multiphasic process in which the secondary structure in α-helices and β-sheets and some turns is formed more rapidly than is the overall folding.

It is desirable to corroborate the results of the novel procedure of detecting and characterizing an intermediate in the refolding of barnase by protein engineering methods[2], with a second technique. The method of choice is the NMR-hydrogen-exchange procedure of Udgaonkar and Baldwin[3] and Roder *et al.*[4] because it can detect individual interactions as well as gross structure. Their procedure is based on the observation that protons that are in $\mathrm{NH}\cdots\mathrm{O{=}C}$ hydrogen bonds are protected to varying extents against exchange with solvent[3,4]. Samples are taken from a reaction mixture in which a deuterated protein is being refolded in D_2O and NMR is used to examine which of the ND groups are in rapid exchange and which are protected. By this means, it was shown that the repeating secondary structure in regions of β-sheet in RNase A (ref. 3) and α-helices in cytochrome *c* (ref. 4) are formed faster than is the final folded structure.

The refolding of barnase was first monitored by the change in the fluorescence of its three tryptophan residues under identical conditions to those used in the NMR experiments (Fig. 1, 1.3 M (deuterated) urea, 99.8% D_2O, pD 6.3 at 25 °C). There is a fast phase accounting for 80% of the reaction, which has a half life of about 140 ms, and a slow phase of 20%, which is 50 times slower with a half life of 8.5 s. The latter phase corresponds to the interconversion from *cis* to *trans* of the fraction of proline residues that equilibrate in the unfolded enzyme—the native enzyme has three proline residues that are all *trans*[1,2]. There is some hint that the fast phase could be resolved into two phases of rate constant 11–16 s^{-1} and 4.6 s^{-1} (Fig. 1).

NMR experiments were made possible by assigning more than 99% of the proton resonances in the ^{1}H NMR spectrum of barnase to their amino-acid residues[5]. Some 45 of the backbone NH protons in the folded protein undergo slow exchange with solvent because they are involved in hydrogen bonds in secondary and tertiary structure. Barnase was denatured and all exchangeable NH groups deuterated (>90% exchange) by incubating in 6.5 M (deuterated) urea and 99.8% D_2O at pD 6.3. The denatured and deuterated protein was allowed to refold by diluting fivefold into D_2O at 25 °C. Samples were taken during the refolding process and exposed for 5–15 s to a labelling pulse of H_2O buffered at pH 8.5 where there is fast exchange of unprotected ND deuterons ($t_{1/2} \sim 5$ ms; refs 3, 4). The pH was lowered to 3.5, where exchange is very slow, and the fraction of H-exchange measured by two-dimensional (2-D) NMR.

It is found (Fig. 2) that 29 of the positions are sufficiently protected under the experimental conditions that the change in protection with time may be followed. Three of the positions, ND(H)s of Ile 25, Asn 77 and Ser 50, are protected according to a time course that is essentially identical to that of the overall refolding process (Fig. 3). Defining the fraction of protons in rapid exchange at zero time as 1.0, compared with 0 when protected in the fully folded protein, the amplitude of the kinetic changes is 0.74–0.76. The theoretical maximum amplitude for the fast phase is 0.8, as 20% of the protein folds slowly, limited by the proline isomerization. Significantly, Ile 25, Asn 77 and Ser 50 make tertiary hydrogen bonds that are not within regular secondary structure but are a consequence of the overall fold of the molecule. It is found, however, that several of the deuterons in the regular secondary structure become protected much faster (Fig. 3 and Table 1). The time courses for many of these are at least biphasic, the slower phase varying from 10–28 s^{-1}, with an amplitude of 50–80% of the maximum. The fastest phase(s) is generally complete by 5 ms and has a smaller amplitude of 10–50%. Multiphasic exponential curves are notoriously difficult to analyse as, unless the data are exquisitely accurate, the sum of two or more exponentials with time constants differing by a factor of two can usually be fitted to a single exponential. But simple inspection of the results shows clearly and unambiguously that extensive secondary structure is formed on a time scale that is shorter than that for the overall folding.

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The question has been raised as to whether the multiphasic kinetics results from a collection of parallel pathways rather than from a sequential mechanism of reaction, that is, the kinetics is simply a consequence of a heterogeneous population of unfolded molecules, each folding at its own rate (equation (1), where ΣE_U is the set of unfolded states that forms the folded protein E_F, by a set of parallel first-order rate constants, Σk). It can be difficult to distinguish

$$\Sigma E_U \xrightarrow{\Sigma k} E_F \tag{1}$$

between parallel and sequential pathways when observing just the overall macroscopic properties of a protein. But this can be done when observing individual sites, as in hydrogen exchange. The kinetics of formation of fully folded protein proceeding by a collection of parallel pathways without any intermediates would be an ensemble of exponential traces each of low amplitude, with the total amplitude being 100%. The same multiexponential time course would apply to the formation of each and every noncovalent bond in the fully folded structure, although the amplitude of the exponential phases would be lower if the noncovalent bond is present to some extent in residual structure in the 'unfolded' state. But we observe a variety of rate constants, varying from clear biphasics on a fast time scale to the tertiary hydrogen bonds involving Ile 25, Asn 77 and Ser 50, which are formed with nearly 100% amplitude of signal in a time course approximating to that of a single exponential. There could be a heterogeneous population of unfolded molecules, but to be consistent with the observed kinetics, they would have to form rapidly a common intermediate before the rate-determining step,

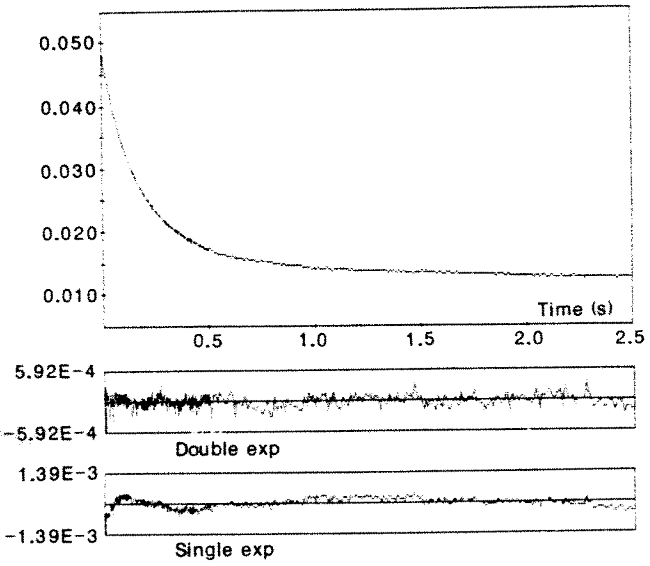

FIG. 1 Time courses for refolding of barnase in D_2O, 100 mM MES buffer (pD 6.3), 1.3 M urea at 25 °C, monitored by regain of tryptophan fluorescence, measured in an Applied Photophysics stopped-flow fluorimeter of deadtime 1 ms with excitation at 290 nm and emission at wavelengths greater than 300 nm selected using a cutoff filter. Protein that was acid-denatured in D_2O at pD 1.8 and 1.3 M deuterated urea was mixed 1:1 with a solution of D_2O, 200 mM MES (pD 6.3) and 1.3 M urea at 25 °C, the concentrations of urea being balanced to eliminate Schlieren effects. Twenty three transients were averaged. The lower two panels show the deviation of the data from theoretical curves fitted to either a single exponential for the faster changes (very bottom) or a double exponential. The data seem to fit slightly better a double exponential of rate constants 11.4 and 4.55 s^{-1}, with amplitudes in the ratio 1:3; than a single of 5.1 s^{-1}—the scatter is smaller and seems to be randomly distributed. Refolding kinetics were also performed with a Perkin-Elmer spectrofluorimeter MPF 44B fitted with a stopped-flow mixer[2]. Excitation was at 290 nm and emission at 315 nm. This machine, with monochromators for both excitation and emission, allows emission to be monitored at a single wavelength and also the mixing of high concentrations of urea with water without Schlieren effects, but it has a dead time of 30 ms. Denatured protein in 6.5 M deuterated urea, D_2O, 50 mM MES (pD 6.3) was diluted 11-fold into D_2O, 0.78 M deuterated urea, 50 mM MES (pD 6.3) to give the same final concentrations as for the NMR experiments. Data (not shown) fit equally well a double exponential of rate constants ~16 and 4.58 s^{-1} or a single of 5.6 s^{-1}. Repetition of the experiments in H_2O at 0 M urea where renaturation is faster, the conditions used in the calculations by Matouschek *et al.*[2], showed that the faster phase is lost in the dead time of the stopped-flow machine and so only the slower phase is observed and accurately measured there.

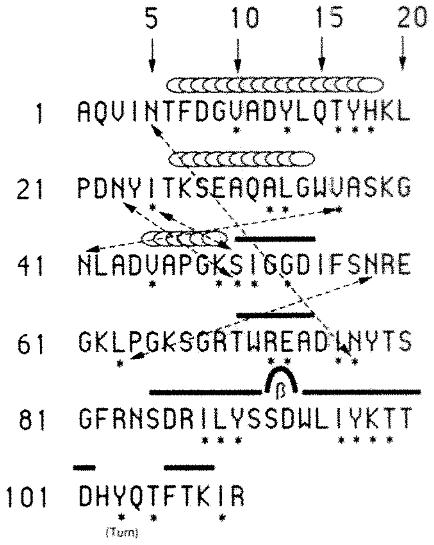

FIG. 2 Backbone >ND(H)s protected from exchange with solvent during refolding. Time courses for the protection of NH groups (ND groups) during the refolding of denatured barnase were determined in 1.3 M deuterated urea, D_2O (pD 6.3) 50 mM MES buffer at 25 °C. Barnase was denatured by incubating in 6.5 M deuterated urea in 99.8% D_2O buffered at pD 6.3 and the NH groups allowed to exchange with the solvent to give ND groups (>90% exchange). The denatured protein was mixed with four volumes of buffered D_2O to initiate refolding at the same pD using modified versions of the quenched flow or pulsed quenched flow apparatus of Fersht and Jakes[6]. After time intervals from 0 (that is, direct refolding in labelling buffer) and then 6—550 ms, samples were exposed to a labelling pulse of four volumes of H_2O buffered at pH 8.5 (100 mM Tris-Cl buffer) where exchange of unprotected ND deuterons is very rapid. After 5–15 s, which is sufficient time for the all-*trans*-prolyl enzyme to fold fully, the pH was lowered to 3.5 with 500 mM ammonium formate, where exchange is very slow. (The 20% of protein that contains *cis* prolines is fully exchanged in the first fraction of a second in the labelling pulse, in which time <1% of the proline residues isomerize.) Each sample was concentrated and the buffer changed to D_2O, pD 3.5, with an Amicon MC-8 ultrafiltration cell. Phase-sensitive COSY spectra were recorded at 27 °C on a Bruker AM 500 spectrophotometer[5]. The intensities of the cross peaks in the fingerprint region of the respective residue were determined by measuring peak to peak heights in cross sections parallel to the ω_2 axis[7]. The peak heights were standardized with reference to the cross peak of the non-exchanging aromatic ring protons of Tyr 13. The reproducibility of the data varies according to peak intensity and the degree of overlap. The worst data points analysed are reproducible to within ±10% of maximum peak height, the majority to about ±5–7%, the error being constant throughout each time course. Control experiments on native protein incubated under the conditions of the labelling pulse for several minutes showed that no detectable exchange occurs at the analysed sites. Equilibrium measurements of the unfolding of barnase in D_2O, deuterated urea, 50 mM MES (pD 6.3) and 25 °C were performed as in H_2O[8], and the free energy of unfolding found to follow the equation $\Delta G_U = (11.38 - 2.27[\text{urea}])$ kcal mol^{-1}. Under the conditions of the NMR experiments the equilibrium constant is calculated to be 10^6:1 in favour of folding.

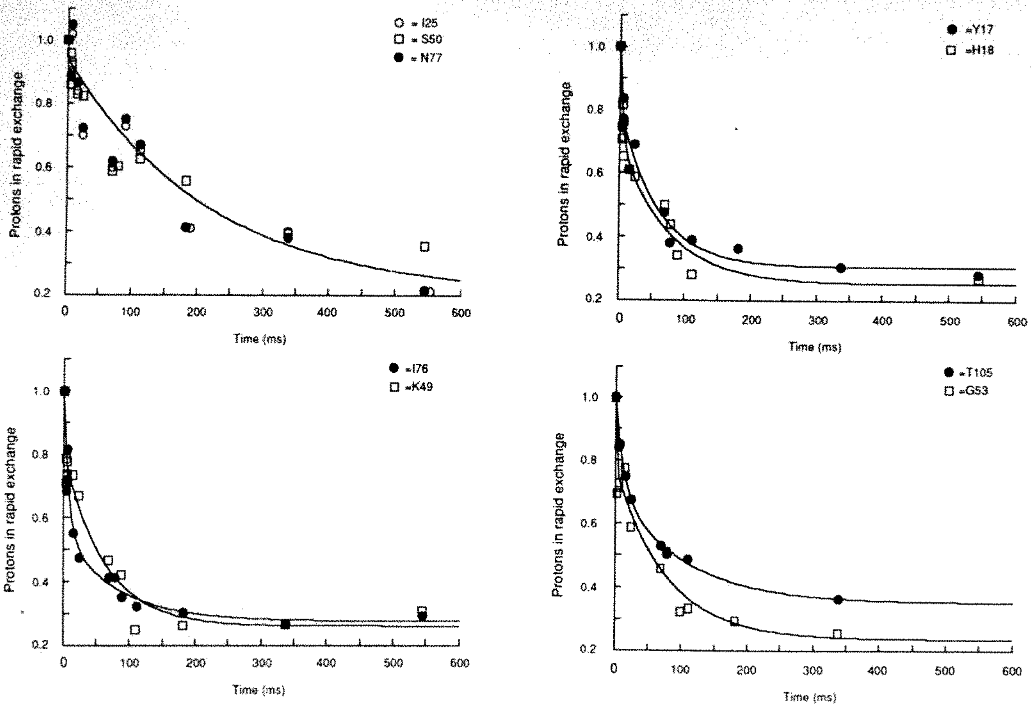

FIG. 3 Time courses for the protection of backbone >ND(H)s against exchange. Conditions as in Fig. 2. Top left, residues Ile 25, Asn77 and Ser50 are protected with a rate constant of 5 s^{-1}, that for the overall folding. The other panels contain representative time courses. The fraction of exchangeable protons is defined as 1.0 at zero time. The expected endpoint for 'complete protection' is 0.2 as 20% of the denatured protein, that fraction containing *cis* prolines, folds with a rate constant of 0.082 s^{-1}, and 550 ms is only 6% of the half life for that step. There is no fast phase in the data for residues Ile25, Asn77 and Ser50 (top left); there is random scatter of time-points compressed in the region 0–7 ms.

TABLE 1 Protection against hydrogen–deuterium exchange

Residue	Location of >NH	Phase 1 Rate constant* (Amplitude)† (s^{-1})	Phase 2 Rate constant* (Amplitude)† (s^{-1})
V10	Helix	>500 (0.31)	28 (0.36)
Y13	Helix	poor data, fast protection	
T16	Helix		18 (0.60)
Y17	Helix	>250 (0.17)	18 (0.52)
H18	Helix	248 (0.29)	14 (0.46)
I25	Tertiary (with S50)		5.8 (0.76)
A32	Helix	poor data, fast protection	
L33	Helix		20 (0.60)
V36	Tertiary (with N41)	>400 (0.27)	3.7 (0.66)
V45	Helix	>400 (0.32)	15 (0.39)
K49	Helix	>500 (0.19)	16 (0.56)
S50	Tertiary (with N23)		4.7 (0.72)
I51	Sheet	>250 (0.22)	13 (0.44)
G53	Sheet	>250 (0.22)	13 (0.54)
L63	turn (with N58)	>250 (0.27)	15 (0.26)
R72	Sheet	>500 (0.52)	15 (0.26)
E73	Sheet	poor data, fast protection	
I76	Sheet	130 (0.44)	12 (0.27)
N77	Tertiary (with N5)		5.8 (0.74)
I88	Sheet		19 (0.53)
L89	Sheet		18 (0.55)
Y90	Sheet		26 (0.48)
I96	Sheet		13 (0.75)
Y97	Sheet	poor data, fast protection	
K98	Sheet		28 (0.60)
T99	Sheet	>500 (0.33)	26 (0.37)
Y103	Turn	250 (0.38)	5.7 (0.49)
T105	end of sheet	80 (0.28)	10 (0.35)
I109	Sheet	>200 (0.19)	10 (0.52)

* The change in protection was analysed by fitting to a two-step exponential of the form $y = A\exp(-k_1 t) + B\exp(-k_2 t) + C$ using the program 'Regression' (Blackwell Scientific Publications, Oxford, UK) which employs the Marquardt algorithm.

† The fraction of protons in fast exchange is defined as 1.0 in the unfolded protein at zero time and in the fully folded protein as 0. The maximum amplitude expected for the phases studied here is 0.8 as 20% of the protein folds on a much longer time scale ($t_{1/2} = 8.5$ s). The detection of a fast phase that is complete in the dead time of the apparatus, hinges crucially on the value of the point taken at zero time. The method of determining the zero time-point seems valid as the zero-time values for the ND groups of residues 25, 50 and 77—which are relatively slowly protected with a simple exponential time course ($t_{1/2} = 140$ ms) or a virtually superimposable double exponential time course (see Fig. 1)—are within experimental error of values extrapolated back from 5–7 ms after mixing. Single-letter notation used for amino-acid residues.

and all the molecules pass through this intermediate (equation (2)). The kinetics thus show that there must be at least one intermediate, E_I, formed during folding.

$$\Sigma E_u \overset{\Sigma k}{\rightleftharpoons} E_I \cdots\longrightarrow\cdots \xrightarrow{k_{slow}} E_F \qquad (2)$$

The simplest explanation of the data is that there is a rapid equilibrium set up in the submillisecond timescale in which many of the backbone ND(H)s are protected. The equilibrium constant, which is related to the amplitude of the fast phase for each residue and the degree of protection against the labelling pulse, is a function of the position in the protein. Whether these are abortive complexes or whether the same bonds as in the final structure are formed is not obvious. The helices and sheets are formed from these complexes with a rate constant of ~15 s^{-1}. Residue Tyr 103, which is in a bend, and Val 36, which makes a tertiary interaction, are residues that are not in the helices or sheet. They exhibit some of the rapid protection and then take up their final interactions with a rate constant equal to that of the final folding step of ~5 s^{-1}. Ile 25, Asn 77 and Ser 50 make their tertiary interactions also at 5 s^{-1} as the structure finally folds.

The NMR H-exchange data confirm the conclusions from the protein engineering studies that the refolding of barnase involves at least one discrete intermediate. Both techniques show that the C-terminal end of the major helix is formed before the main transition state for folding. The NMR data show further structural features, such as early formation of sheet, and definite indications of earlier folding intermediates before the one observed in the protein engineering studies. ☐

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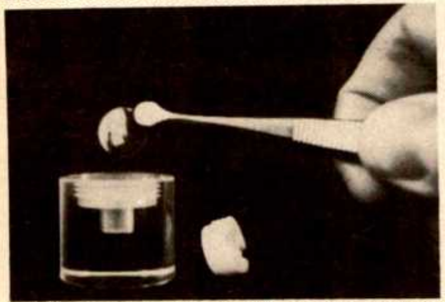
New product news

Products for the midsummer season include magnetic beads for the isolation of poly (A)$^+$ RNA, a mycoplasma testing service and plate-tectonic modelling software for geology buffs.

Genzomed supplies a **microdialysis system** — µ-DIA — for use with small sample volumes (50–200 µl) of proteins and other macromolecules *(Reader Service No. 101)*. The $25 (US) µ-DIA can be used for desalting, buffer exchange, separation of excess radiolabel, enzyme assays, and the purification of proteins, nucleic acids and other macromolecules. It is available with pre-cut membranes with molecular weight cut-offs of between 500 and 500,000 Da. All kinds of bacteria filters, membrane ultra filters, blotting membranes and dialysis membranes can be used with µ-DIA. Dialysis takes 1–2 hours, Genzomed says, depending on the number of samples and the buffer.

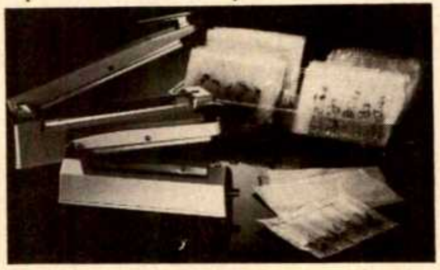


µ-DIA: Genzomed's small sample volume microdialysis system.

ACCU-SPERM is an **acrosin activity assay** from O.E.M. Concepts that can be used to study subfertility and infertility and which may aid in the evaluation of sperm function *(Reader Service No. 102)*. Acrosin can occur in spermatozoa in both inactive and active forms. Proacrosin, the inactive form, represents about 90 per cent of the acrosin in human spermatozoa. The assay uses a membrane-based separation of sperm cells from seminal fluid. At basic pH, proacrosin is converted to enzymatically active acrosin and the total acrosin activity of human spermatozoa is determined. Results are obtained in 90 minutes, says O.E.M., and are read spectrophotometrically by measuring absorbance at 405–410 nm. The $450 (US) ACCU-SPERM assay kit contains sufficient reagents for 48 assays.

AutoGen 540 is the latest benchtop system for the **isolation of DNA and RNA** from a wide variety of biological sources *(Reader Service No. 103)*. The instrument is designed for the rapid isolation of plasmids, cosmids, phage DNA, genomic DNA and RNA from bacterial cultures, tissue suspensions, blood and plant cell preparations. Two versions are available. The $36,000 (US) basic version, which has nine reagent positions and is supplied with ten different DNA and RNA protocols on the system's floppy disk. Applications protocols are continually being developed and updated by AutoGen. The more advanced $42,500 (US) programmable version has 12 reagent positions and, in addition to the ten standard protocols, is fully programmable through an MS-DOS based PC system. According to AutoGen, both versions can process up to 12 samples simultaneously in cycle times of less than 40 minutes. The instrument may be of interest to researchers in the fields of genetic engineering, DNA sequencing, mapping, analysis and as a sample preparation device for DNA probe diagnostics.

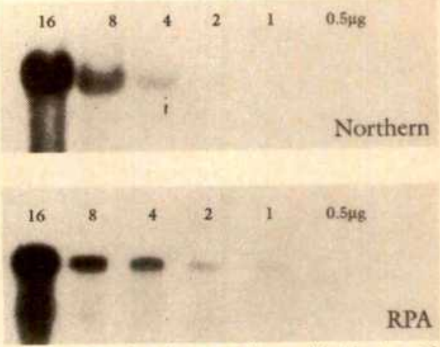
Plant micropropagation is a new area for Becton Dickinson and one of the first products off the production line is the Falcon CultuSAK for plant tissue culture *(Reader Service No. 104)*. Designed to replace conventional plant tissue culture equipment, CultuSAKs are made of non-porous translucent film that is permeable to gases but, says Becton Dickinson, virtually impermeable to liquids. The system can be sealed completely to reduce the risk of contamination and the problems of desiccation of the culture media. CultuSAKs are available in four sizes to support different types of plant growth.



Bagged up and ready to grow.

RNA research

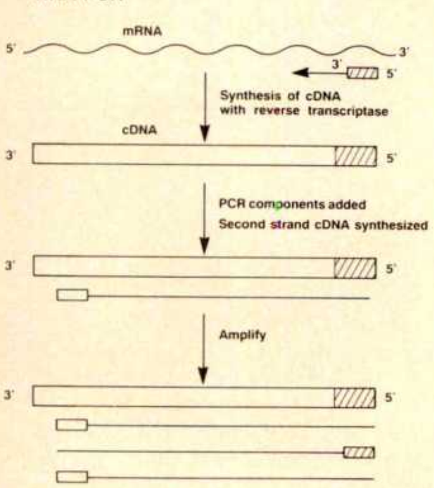
According to Ambion, its Ribonuclease Protection Assay (RPA) kit for the **detection and quantitation of mRNA** is at least ten-times more sensitive than northern blotting *(Reader Service No. 105)*. Using high specific activity probes, it is possible to detect as little as 100 femtograms of target RNA after a three-day exposure to X-ray film. Because of the high sensitivity of the assay, low abundance transcripts can be detected without poly(A) selection. Ambion attributes the increased sensitivity of its RPA kits to the following: the hybridization reaction occurs in solution, whereas target RNA is bound to a filter with northern blotting; and after RNase digestion, the protected labelled material is separated from digested material by gel electrophoresis, which increases the signal-to-noise ratio. Ambion's $195 (US) RPA kit contains enough reagents and positive controls for 120 assays. The company also provides *in vitro* transcription kits for RPA probe synthesis using SP6, T7 or T3 RNA polymerases.



RNA detection by northern blotting and Ambion's RPA kit. Exposures were 64 hours and 6.4 hours, respectively.

Dynal offers Dynabeads Oligo (dT)$_{25}$ **magnetic beads for the isolation of poly(A)$^+$ RNA** directly from solid tissue, cell lines and plants *(Reader Service No. 106)*. The solid phase technology of Dynabeads Oligo (dT)$_{25}$ eliminates the need for oligo (dT) cellulose columns. Pure cytoplasmic mRNA can be isolated from cell suspensions in 15 minutes, says Dynal. Recovery rates for mRNA of greater than 90 per cent are possible, while the fast reaction kinetics reduce the risks of physical and enzymatic degradation. mRNA isolated in this way can be used for northern blotting, cDNA synthesis, *in vitro* translation experiments, or as starting material for PCR reactions. Dynabeads Oligo (dT)$_{25}$ can be supplied in a $190 (US) ready-to-use kit form, or as a $500 (US) single reagent (5 × 1 ml containing 5 mg beads per ml, where about 2 µg mRNA is isolated per mg beads). Optimal results are obtained when using Dynal's magnetic particle concentrator.

All you need for **RNA transcription and cDNA amplification** in a single tube is provided by the new GeneAmp **RNA PCR kit** from Perkin-Elmer *(Reader Service No. 107)*. The kit can be used to generate a cDNA product in excess of 2 Kb from all types of RNA (mRNA, tRNA, rRNA, total RNA and viruses) and to detect specific RNA species present at low copy number, says Perkin-Elmer. Reagents are supplied premixed and optimized for reverse transcription of RNA and subsequent amplification of the resulting cDNA products using the GeneAmp PCR process. Each kit includes the GeneAmp PCR core reagents, a positive control RNA template transcribed from the plasmid pAW 109, control primers that flank an Il-2 alpha sequence in the control RNA, random hexamers, reverse transcriptase, RNase inhibitor, dATP, dTTP, dCTP and dGTP. Sufficient positive control RNA is provided for 25 reverse transcription/amplification reactions starting with 10^4 copies per reaction.


RNA-PCR
mRNA
5'
3'
3'
5'
Synthesis of cDNA with reverse transcriptase
cDNA
3'
5'
PCR components added
Second strand cDNA synthesized
3'
5'
Amplify
3'
5'

GeneAmp RNA PCR kit.

Products for PCR

Clontech offers **PCR primers** for use in the GeneAmp PCR process for the amplification of DNA inserts contained in lambda vectors *(Reader Service No. 108)*. Insert screening amplimers are available for lambda gt10, lambda gt11, lambda gt22, SWAJ-2, SWAJ-4, lambda BlueMid and lambda Zap vectors. Clontech's amplimers can be used to determine the size of the DNA inserts or to recover inserts that cannot be excised from the vector. Researchers pick a recombinant plaque, freeze/thaw it briefly, and then amplify the insert by PCR using the screening amplimers and the GeneAmp PCR reaction kit. The amplification product can be analysed on a gel, cloned into another vector, or sequenced. Each insert screening amplimer consists of a pair of primers and a detailed protocol.

The OptiTaq kit from Bios makes light work of **determining the optimal buffer to use with specific primer:target DNA pairs** in a PCR experiment *(Reader Service No. 109)*. Excessive or insufficient amounts of magnesium can interfere with the specificity and yield of PCR results, says Bios. The magnesium content required for a specific primer:target DNA pair will also vary according to the source of *Taq* polymerase and the method of preparation and/or storage of the DNA template. The kit includes 1 ml each of ten different 10× buffers with varying concentrations of $MgCl_2$ (5 mM to 50 mM). A series of reactions using each of the OptiTaq buffers are set up for each primer:target DNA pair. Normal thermocycling procedures are followed to produce PCR products. An aliquot from each series of reactions is run on an agarose gel and the buffer yielding the the greatest amount of specific product is determined.

OptiTaq kit for PCR — all you need to optimize buffer conditions.

Protein isolation/purification

Rainin Instruments has introduced a new **protein fractionator** — the Model RF3 — which is designed to provide gram-scale purification of proteins in 2–4 hours *(Reader Service No. 110)*. Separation occurs by isoelectric focusing in free solution, without gels, solid supports or mesh separators, and operation is unaffected by isoelectric precipitation. With the RF3, the sample solution flows rapidly through a thin-channel electrophoretic cell; this set up provides a highly stable laminar flow that prevents convective mixing, says Rainin. Short residence times and an efficient heat exchange system maintain controlled operating conditions. A 30-tube fraction collector is supplied with the Model RF3.

The **ultrafast microprotein analyser** (UMA) from Michrom BioResources is a fully integrated microbore HPLC-based system designed for specialized applications *(Reader Service No. 111)*. The system consists of a binary gradient microbore solvent delivery system, a 10-port automated sample injector, a thermally-controlled mobile phase column and flow-cell system, UV/visible detector with custom micro flow cell, and a PC/AT controller/data system. Two applications packages are available. The first, for the real-time monitoring of peptides and proteins produced either synthetically or by recombinant DNA techniques. The second, designed for the micropreparative purification and isolation of peptides and proteins at the 5–1,000-pmol level. Michrom says that recoveries of greater than 90 per cent for peptides and proteins are possible at the 5 pmol level, with collection volumes of 10–25 μl.

Cell culture

Mycoplasma Experience offers a complete **mycoplasma testing service** for the detection of mycoplasma contamination in cell cultures, sera, vaccines and biologicals *(Reader Service No. 112)*. The company says its own brand of recovery media allows for the rapid growth of common cell culture contaminants, including strains that cannot be grown on conventional mycoplasma media. Mycoplasma Experience offers full technical support, confidential consultations and contract research and development, as well as a cost-effective voucher scheme for regular customers.

For high-volume culture work, New Brunswick Scientific has introduced the PourMatic carousel-type, self-stacking, **automatic agar dispenser** *(Reader Service No. 113)*. PourMatic can pour and stack up to 320 petri dishes in 20 minutes, says NBS. The instrument can be set to dispense between 5 and 20 ml of agar in 1-ml increments and with an accuracy of ± 5 per cent, says NBS. A complete and compact plate preparation system can be put together by interfacing the PourMatic to a sterilizer. This set up is designed to both save time and effort in the laboratory and ensure that the agar plates are of a consistent quality with virtually no contamination.

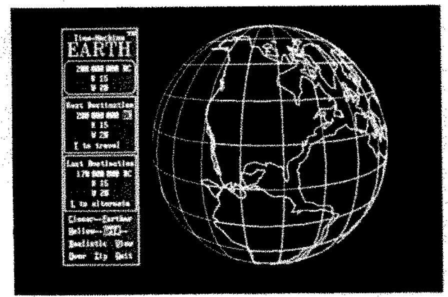
Measuring 10.5 inches in diameter and 13.25 inches in height, the Ritter M-8 EasyClave self-contained **steam sterilizer** from Midmark is designed to be lightweight, portable and useful in laboratories where space is at a premium *(Reader Service No. 114)*. No plumbing is required. To operate, the user must fill the sterilizer with distilled water, insert the instruments, close the lid and start. Sterilization cycle times take 25 minutes, says Midmark. Safety features include a self-

Steam clean with the M-8 EasyClave.

locking cover, pressure control valves and a low-water indicator system.

Software smarts

Time-Machine Earth is software from Sageware that is designed for **plate-tectonic modelling** on an IBM PC or compatible *(Reader Service No. 115)*. Two versions are available to meet the differing needs of users: a personal edition that can be used as a teaching tool and a more sophisticated professional edition that, although it has many features in common with the personal edition, is aimed at a research level. Time-Machine Earth simulates the geological history of the planet and displays computer-generated satellite images of the globe showing the positions of the oceans and continents at a selected time period. According to Sageware, users can look at the earth up to 550 million years ago or 40 million years into the future with accuracy. A speculative look can be taken at the earth up to 2,000 million years in the past or future. Teachers can use the $69.95 (US) personal version to demonstrate the fundamentals of continental drift. Using the 'blink' comparison, users can move between two eras with a keystroke. Alternatively, time periods can be superimposed. The $245 (US) professional version allows the user to change the placement of continents in order to create maps based on alternate theories of continental movement. Each land mass can have its own independent pole and angle of rotation.



Time-Machine Earth software brings plate-tectonic modelling to PCs.

Watermaze, developed by Edinburgh-based Watermaze Software and available from Paul Fray, is **software for automatically tracking and analysing the path of a swimming rat** *(Reader Service No. 116)*. The software, which runs on a 32-bit Acorn Archimedes computer, provides a simple means of monitoring the cognitive effects of various drugs and can be used as a tool for basic research involving spatial learning. Animals are monitored via a ceiling-mounted video camera coupled to an image analysing device and computer. An online interpolation routine smooths occasional tracking errors that can occur when the rat swims underwater. The path taken, together with the interpolated values, are displayed in real time and can be printed or stored on disk.

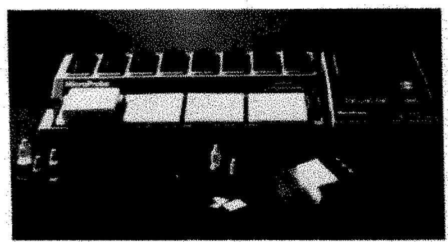
Mix it

The gentle two-dimensional action of the Robbins Scientific **mixer**, which is available from Nycomed, is designed to mix thoroughly solutions or blood-cell suspensions *(Reader Service No. 117)*. As tubes are both rotated at 18 r.p.m. and rocked up and down, the mixer is particularly useful for keeping insoluble materials, such as separating beads, in suspension. Two models accommodating twelve 10–17-mm tubes or eight 50-ml tubes are available. The mixer heads are interchangeable so that both sizes of tube can be used.

Jencons Scientific's **four-plate orbital shaker**, which is designed for mixing without cell disruption, is a handy benchtop aid *(Reader Service No. 118)*. The microplate mixer, costing less than £350 (UK), has a shaking speed that can be set to between 0 and 1,800 r.p.m. An optional extra is the 1–30-minute timer.

Benchtop aids

The MicroProbe **manual slide stainer** from Fisher Scientific is designed to provide greater uniformity, reproducibility and speed to chemical staining, immunohistochemical and *in situ* hybridization procedures *(Reader Service No. 119)*. The $2,595 (US) MicroProbe system, with its 10 × 26-inch footprint, has three major components: a slide holder, staining module and incubator. The slide holder has slots for up to ten pairs of special slides and so 20 specimens can be processed simultaneously. The gap between each slide pair, which fills by capillary action, can accommodate 5-μ tissue sections.



MicroProbe: Fisher Scientific's all-in-one slide stainer and incubator.

The instrument's staining module has eight stations for bulk-type dewaxing and staining reagents and four stations for use with more expensive immunoreagents, DNA probes or blotting pads. User-programmable temperature control from 30 to 110 °C, with an accuracy of ± 0.5 °C, is possible with the system's microprocessor-controlled incubator.

With safety precautions in mind, Owl Scientific has a **radiation safety rack** for storing tubes containing beta-emitting isotopes *(Reader Service No. 120)*. The $49 (US) rack box comes with an 80-slot microtube rack, but can also accommodate scissors, markers and other small laboratory utensils. It is constructed of

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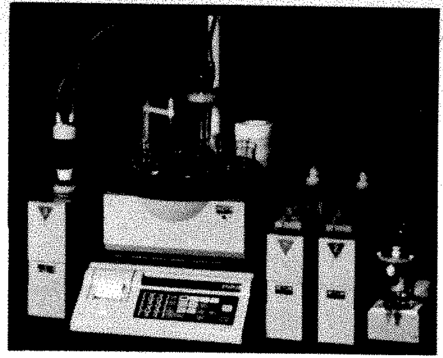
clear, heavy-duty 0.375-inch thick acrylic and measures 9.75×3.375×3.25 inches. The box is equipped with a tight-fitting lid and four 'non-skid' rubber feet. Custom box designs are available on request.

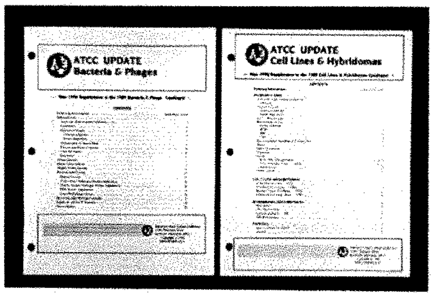
Mitsubishi Kasei has launched the compact Model GT-06 **automatic titrator**, which can be configured to perform all types of titrations — acid-base, redox, argentmetric, chelatemetric, non-aqueous, or photometric *(Reader Service No. 121)*. The basic system comes with a standard built-in printer and RS232 interface for linking it up to a balance, and with an optional bi-directional computer communication. For ease and speed of operation, up to eight titration files can be stored in the systems's memory. When the Model GT-06 is linked to the Model GT-LD photometric detector, users can perform photometric and chelatemetric titrations, and titrations using an indicator. Another option is the Karl Fisher titration unit.


Model GT-06 modular titration system from Mitsubishi Kasei.

Information sources

Scientists involved in connective tissue and blood anticoagulant research may be interested in the **1990 biochemical catalogue of leech-derived products** from Biopharm *(Reader Service No. 122)*. The catalogue features a host of enzyme and protease inhibitors, including hirudin (a potent inhibitor of thrombin), leech collagenase (a collagen-specific enzyme), leech hyaluronidase (an endo-β-glucuronidase) and hementin (a fibrinogenolytic enzyme).

Keep up to date with the latest **catalogue updates** from the American Type Culture Collection (ATCC) *(Reader Service No. 123)*. The 38-page update to ATCC's 1989 bacteria and bacteriophages catalogue includes information on 450 new strains, recommended procedures for reviving preserved cultures, methods for storing cultures, and media formulations for use in subculturing each strain. In addition, an index of about 650 strain designations in use by other laboratories and culture collections should provide a useful aid in the location of a particular strain. The 53-page cell line and hybridoma update to ATCC's 1988 catalogue lists an additional 80 cell lines and 170 hybridomas. An index to the antigenic determinants that react with the mAbs produced by the hybridomas and a species/tissue index to the cell lines are included. Copies of both updates are free on request and are available via ATCC's online computer service.



ATCC's catalogue updates — now free on request or online.

Have you ever had cause to look for an organism with very precise properties? Yes, well the Institute of Biotechnology's **24-hour online information service** — the Microbial Strain Data Network (MSDN) — for yeasts, algae, bacteria, cell lines, hybridomas, recombinant clones and libraries may be the answer *(Reader Service No. 124)*. The MSDN combines a directory to information services with related databases — catalogues, primary laboratory data and other information centres. As the information service is linked up to an electronic mail service, users can place orders without exiting the system. The service is available online or through the MSDN Secretariat and costs £25 (UK) for registration and documentation: subsequent charges are made for online use. A typical five-minute search during prime time would cost about £2.15 (UK), says IOB. In addition, MSDN and DATASTAR have entered into an agreement to provide an electronic gateway between the two services. DATASTAR is offered at a discounted rate to MSDN users. This service provides access to databases in biomedicine, chemistry and biotechnology, including Medline, Scisearch, Biosis, Chemical Abstracts, Current Biotechnology Abstracts, Computer Database, and Excerpta Medica.

An extensive range of laboratory consumables and equipment for **sample handling** tasks are listed in the new Pierce and Warriner catalogue *(Reader Service No. 125)*. A vast array of vials, ampoules, flasks, syringes, closures, seals, discs and septa are featured in the free catalogue. Also included are stirrers, heaters and evaporators, as well as equipment for filtration and dialysis.

Sigma has a line of **gold conjugates** which in analytical procedures provide an alternative to RIAs and ELISAs (*Reader Service No. 126*). Greater than 50 products are offered in three complementary/interactive systems: avidin/biotin; lectin/glycoprotein; primary and secondary antibodies/Protein A and G.

These notes are compiled by Diane Gershon from information provided by the manufacturers. To obtain further details, use the reader service card bound inside the journal. Prices quoted are sometimes nominal, and apply only within the country indicated.

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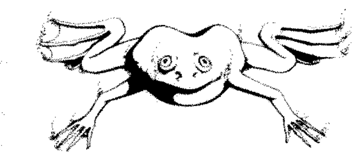
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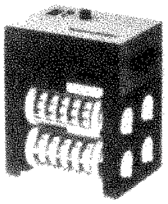
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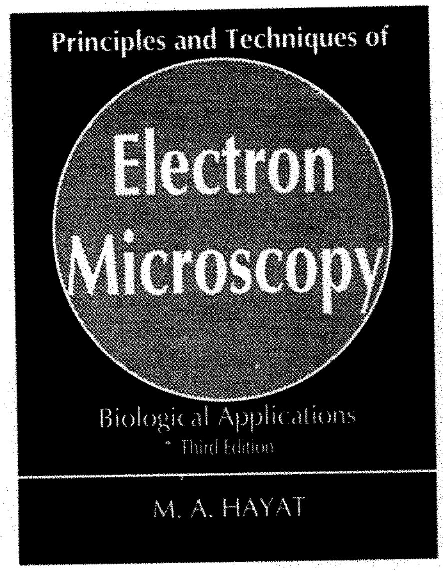

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The Hematology/Oncology Research Laboratory of Dr. Jerome E. Groopman, New England Deaconess Hospital

is initiating a search for a Postdoctoral Fellow in Human retrovirology. Areas of interest include virus-receptor interactions, cytopathic mechanisms, viral tropism, and novel antiviral strategies.

New England Deaconess Hospital is a major Harvard Medical School affiliated institution and a center for AIDS and immunology research. Appropriate salary and benefits are includes in the fellowship.

Applicants should send a C.V., and outline of research interests, and the names of three referees to Dr. Jerome E. Groopman, attn. Dr. Randal Byrn, New England Deaconess Hospital, 110 Francis Street, Boston, MA 02215.

(NW5593)A

LEUKAEMIA RESEARCH FUND
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POST-DOCTORAL SCIENTIST
London SW3

A position, funded by the Leukaemia Research Fund, is available in the Membrane Biology Team for a post doctoral scientist to study the regulation of endocytic membrane trafficking in lymphoid cells. Candidates should have experience in cell and/or molecular biology, and knowledge of immunology or virology will be an advantage. The position is for three years and salary will be £14,031 — £17,022 pa (pay award pending) according to experience. Further details can be obtained from Dr Mark Marsh at the Chester Beatty Laboratories (071-352 8133).

To apply, send a curriculum vitae, in duplicate, with the names and addresses of two referees to the Personnel Officer, Institute of Cancer Research, 17A Onslow Gardens, London SW7 3AL, quoting ref. no. 8.90.S.N.23.

Applicants are advised that smoking is prohibited in the majority of the Institute's premises.

(4339)A

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the *Stockholm Environment Institute (SEI)*

(International Institute for Environmental Technology and Management)

APPOINTMENT OF DIRECTOR
(based in Stockholm)

Applications are invited for the above post on an international basis (non-Swedish citizens) from persons with a secure international research reputation and a wide range of contacts in the programme areas of the Institute detailed below. Sensitivity to environment-development issues in industrialized and developing countries worldwide, preparedness to travel widely in order to develop further the Institute's existing international R&D and information networks and complete fluency in both spoken and written English are all essential requirements. Experience in managing an international, research-based operation in the public or private sector, is also necessary. An appointment will be made for up to four years in the first instance, with the option of a further four years, by mutual agreement. Salary arrangements are negotiable, based on UN criteria.

THE INSTITUTE:

The SEI, established in 1989 following a Swedish Government initiative, is an independent Foundation governed by a Board of fiteen members drawn from developing and industrialized countries worldwide. Substantial core-funding to the SEI Foundation from the Swedish Government for the current phase of operations (up to 30 June 1993) is expected to continue beyond 1993. Additionally, the Institute currently has further funding from a range of external sources.

The Institute's primary objective is to carry out authoritative, policy-orientated studies and research on the assessment and development of technologies in relation to environmental management, and its methods and strategies for promoting sustainable development. It has already established a secure international reputation in these fields.

THE PROGRAMME:

The Board has set guidelines for a rolling-programme of action with the following priority themes: (1) Agro-biotechnological biosafety; (2) Climatic change assessment; (3) Energy futures; (4) Economics, ethics and environmental value. Supporting measures for these priorities include: a) integrated pollutant management strategies; b) water demand/supply management; c) sustainable rural-development; d) dissemination of environmental management capacity.

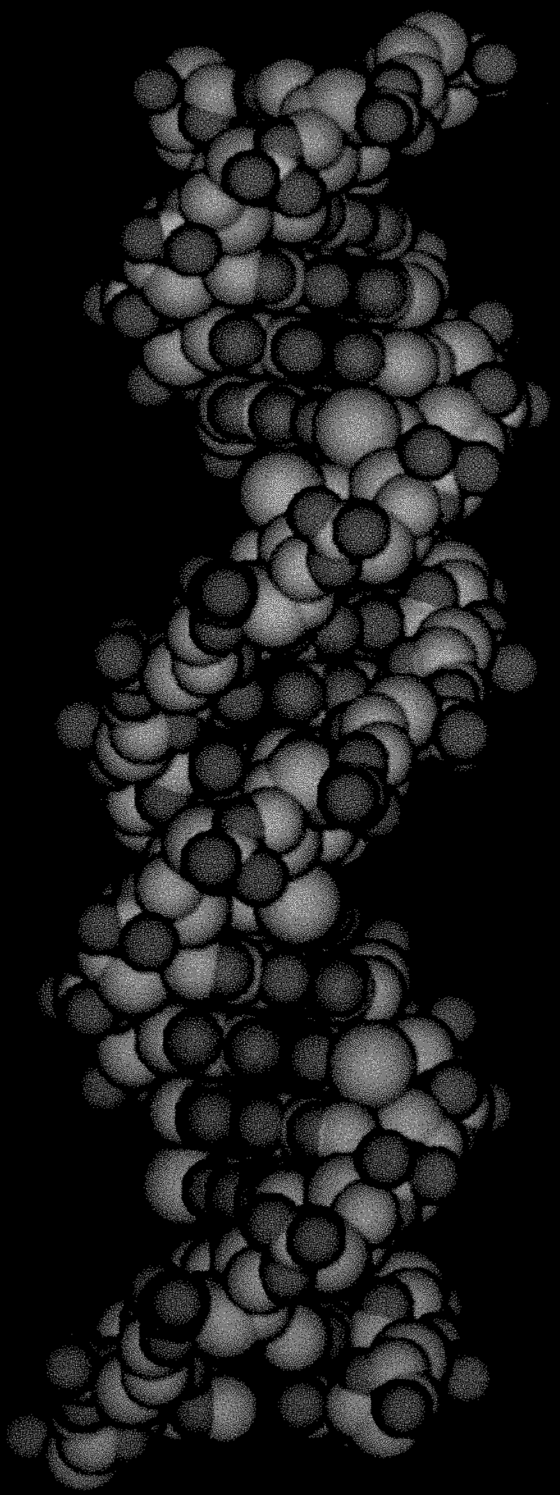
The Institute has good working linkages with similar institutes abroad and with intergovernmental agencies. The SEI already plays a significant role in supporting the secretariat of the UN Conference on Environment and Development (UNCED) during the current preparatory phase, for the Conference in Brazil (June 1992).

The above programme is carried out by a worldwide network of about 70 full-time, part-time, and affiliated field staff and consultants, who collaborate with cognate institutions worldwide and are linked to the SEI Centres in Boston (USA) and York (UK) and with the Headquarters, located centrally in Stockholm. It has been initiated by the present Director (Professor Gordon Goodman) who was responsible for the start-up of the Institute and who retires at the end of 1990.

Applications should be sent not later than 10 September 1990 to the **Chairman of the SEI Board, Professor Gunnar Brodin, Chancellor of the Swedish Universities, P.O. Box 45501, 104 30 Stockholm, telephone +46-8-728 3600 or Kai Olsson, PA Consulting Group, Box 7813, 103 96 Stockholm, telephone +46-8-24 90 20,** from whom further information may be obtained.

(W7600)A

Antisense



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It is expected that suitably qualified candidates will have a proven track record of achievement. If your experience is varied, so much the better. There is plenty of scope for innovation in the pursuit of therapeutics. You will be encouraged in original thought, provided with the resource to turn ideas into action and given the freedom to publish basic research results.

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Applications (including CV and names of two referees) should be sent to Dr S C Bell, Department of Obstetrics & Gynaecology, University of Leicester, University Road, Leicester LE1 7RH from whom further information may be obtained (Tel: 0533 523162).

(4330)A

UNIVERSITY COLLEGE DUBLIN
DEPARTMENT OF ZOOLOGY

Applications are invited for a full-time, academic appointment in the above Department. The apppointment will be initially for a three-year period with the possibility of a permanent position.

Preference will be given to candidates who have a relevant Ph.D. degree, post-doctoral and/or teaching experience and be committed to a productive research programme and excellence in teaching. Research experience in an experimental area of Zoology such as developmental biology, animal physiology or ecotoxicology would be an advantage but applications from scientists working in other relevant areas will also be considered.

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Application procedure: Prior to application, application forms and further particulars relating to these posts may be obtained from the Personnel Officer, University College Dublin, Belfield, Dublin 4. Telephone enquiries: (0001) 693244 ext. 1413/1412. Fax: (0001) 694409.

Intending candidates will be required to submit a curriculum vitae (six copies) together with the names of three referees and the application form. The closing date for receipt of applications is Thursday, 13th September, 1990. (4312)A

THE HEBREW UNIVERSITY OF JERUSALEM
FACULTY OF AGRICULTURE

The Department of Biochemistry and Human Nutrition, Division of Food Science, Faculty of Agriculture, The Hebrew University of Jerusalem, Rehovot invites applications for a tenure track position to carry out research and teaching in Food Science (Food Biotechnology and Microbiology, Food Safety). The position also requires supervision of M.Sc. and Ph.D. students. Language of instruction is Hebrew. The candidate will be expected to teach undergraduate courses in Food Microbiology and Food Safety and suggest graduate courses in the area of his/her competence. The applicant will be expected to develop research programmes and will be required to seek grant support for research purposes.

Applicants should submit résumé, names of at least three references and a brief description of experience and scientific interest to:

Professor Arieh Gertler, Chairman, Department of Biochemistry and Human Nutrition, Faculty of Agriculture, The Hebrew University of Jerusalem, P.O. Box 12, Rehovot 76100, Israel, before 13th October, 1990. (W7599)A

KING'S COLLEGE LONDON
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Applications are invited for the above post. Applicants who are skilled in recombinant DNA techniques inplant biology, that will complement existing work which includes studies of photosynthetic systems, the developmental biology of seeds, secondary product formation, plant/microbe interactions and other aspects of plant biotechnology are encouraged to apply. The successful applicant will be expected to commence on 1st October, 1990 or as soon as possible after this date. Salary will be on the Lecturer 'A' scale currently £10,458-£15,372 p.a. (under review) £1,767 p.a. plus London Allowance.
Further particulars and application forms can be obtained from Professor P.J. Peterson, Head of Division of Biosphere Sciences, King's College London, Campden Hill Road, London W8 7AH. Tel: 071-333 4327.
The closing date for applications is 31st August and interviews are anticipated during the week of 17th September, 1990. (4301)A

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(W7601)A

UNIVERSITY OF EDINBURGH

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Applications are invited from suitably qualified candidates for the William Dick Chairs of Veterinary Clinical Studies in the Faculty of Veterinary Medicine, two of which are currently vacant. The apppointments will be made in two of the three broadly defined areas of veterinary medicine and allied biomedical sciences: Farm Animal, Equine and Small Animal. It is hoped that the successful candidates may be able to take up the appointment from 1st April 1991. The salary will be in the professorial range.

Further particulars may be obtained from the Secretary to the University, University of Edinburgh, 63 South Bridge, Edinburgh EH1 1LS, with whom applications (14 copies), including curriculum vitae and the names of three referees, should be lodged not later than 5th October 1990. Overseas candidates need submit only one copy of the application.

Please quote reference no. 90/90.

(4297)A

EMBL

The European Molecular Biology Laboratory, an international research organization situated in Heidelberg, West Germany, has vacancies for:

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EMBL is expanding its activities in computational biology. The Biological Structures and Biocomputing Prgramme is seeking candidates for several Group Leader positions from scientists with experience in computational molecular modelling and/or molecular dynamics. The successful applicants will develop their own research programmes and also undertake research in collaboration with other scientists at EMBL. Preference will be given to applicants demonstrating an interest in the biological aspects of the problems proposed.

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Candidates will be invited to visit the Laboratory in Heidelberg in the coming weeks for discussions and to give a seminar. Please send a C.V., a brief research proposal, and a list of relevant publications, together with dates when it would be suitable for you to visit EMBL, your telephone and Fax numbers to the Personnel Section.

Applicants should contact Dr. S. Fuller or Dr. D. Suck, Biological Structures and Biocomputing Programme for details.

Closing date for applications: 30.09.1990

Please quote ref. no. 90/31

EMBL
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Postfach 10.2209
D-6900 Heidelberg
Federal Republic
of Germany

(W7606)A

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Division of Medical & Molecular Genetics

RESEARCH ASSISTANT 1B

Guy's Campus

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For further information contact Professor F. Giannelli (071-955 4450) or Dr D. Bentley (071-955 4645).

A full curriculum vitae together with the names of 3 referees should be sent to The Administrator, Division of Medical and Molecular Genetics, 8th Floor, Guy's Tower, Guy's Hospital, London SE1 9RT, by Friday 17th August.

(4310)A

UNIVERSITY OF CALIFORNIA, SAN FRANCISCO AND THE HOWARD HUGHES MEDICAL INSTITUTE

TRANSGENIC MOUSE TECHNICIAN

Experienced laboratory technician wanted to join active group studying development of the immune system. The project involves the generation and analysis of transgenic and chimeric mice by standard microinjection procedures. Experience in animal husbandry, molecular biology and cell culture procedures is preferred, but the emphasis will be placed on finding an individual who is self-motivated and highly responsible. The position is within the HHMI unit at UCSF. Payscale is flexible and commensurate with previous experience. The HHMI is an equal opportunity employer. Please address inquiries to: **Dan R. Littman, MD, PhD, Department of Microbiology and Immunology, Box 0414, University of California, San Francisco, San Francisco, CA 94143.**

(NW5583)A

OPPORTUNITIES IN PROTEIN ENGINEERING

AFRC Reading Laboratory

ORGANIC CHEMIST (Ref. 54/89)

The person appointed will lead organic chemistry research within the Protein Engineering Department.

Close interaction with all other sections of the department will be actively encouraged and a comprehensive programme of synthetic organic chemistry will have to be built up.

The compounds produced will be used to help to evaluate the structure/function relationships of proteins (particularly enzymes).

The chemist will aid the choice of active site chemistry for design of novel biocatalysts.

Qualifications First or upper second class Honours Degree in Organic Chemistry with at least 2 years' post graduate research experience.

Salary on the scale £10,678–£14,909 with performance related increments to £18,139.

BIOCHEMIST (Ref. 26/90)

A biochemist is required to join a group of biologists and biomolecular computing scientists involved in the design and mutagenesis of enzymes and binding proteins. The Protein Engineering Department maintains close working links with other Agricultural and Food Research Council Laboratories with parallel interests, as well as with leading university groups. The biochemist will take responsibility for the biochemical and biophysical characterization of novel proteins within the Enzyme Biochemistry section, by co-ordinating and further developing spectrophotometric, calorimetric, fluorimetric and immunological techniques.

The postholder will also be encouraged to collaborate with crystallographers and other specialists in biophysics, in relating detailed structures of new proteins to their function.

Qualifications First or upper second class Honours Degree in Biochemistry with at least 2 years' post graduate research experience.

Salary on the scale £10,678–£14,909 with performance related increments to £18,139.

POSTGRADUATE STUDENTSHIP NOD0 Protein (Ref. AFRC/PWG)

The *Rhizobium leguminosarum* nodulation gene *nod0* encodes a Ca++ binding protein that shows marked similarity with the *E. coli* haemolysin and other secreted proteins that interact with eukaryotic membranes (Economou *et al.* EMBO J 9:349–354). The NOD0 protein is exported without cleavage of an N terminal leader peptide and is involved in signal transduction between the plant and bacteria. The region of homology between Nod0 and the haemolysin group of proteins consists of a series of tandem nonapeptide repeats which are thought to constitute a novel type of Ca^2 binding domain.

The succesful applicant will be involved in an integrated programme (including molecular biology and protein crystallography) aimed at determining the 3-D-structure of the Nod0 protein. The student will be working with Dr P Goodenough at Protein Engineering Dept, AFRC, Reading Laboratory, will be registered for a PhD. at University of Reading and will have the opportunity to visit and collaborate with the group of Dr J A Downie at John Innes Centre of Plant Science Research at Norwich.

Qualifications Candidates will hold a first or upper second class Honours Degree in Biochemistry, Chemistry, Physics or a related subject.

POSTGRADUATE STUDENTSHIP The Design and NMR Analysis of Novel Protein Molecules (Ref. AFRC/RP)

This project involves designing novel protein molecules based on knowledge of existing structures as revealed by X-ray and NMR analysis. This will involve developing, testing and applying new computational tools.

The designed proteins will be constructed by other members of the department and the resulting protein analysed using NMR and X-ray methods.

The candidates will receive training in protein design and NMR and X-ray analysis of proteins. This work will further elucidate the relationship between protein sequence and protein structure and protein structure and action.

Qualifications Candidates will hold a first or upper second class Honours Degree in Biochemistry, Chemistry, Physics or a related subject. Experience of using computers and computer programming would be advantageous. The ideal candidate would have knowledge of protein structure, NMR and X-ray analysis of proteins.

Non contributory superannuation scheme for the Organic Chemist and Biochemist posts.

Relocation expenses of up to £5,000 may be payable for certain posts.

Further particulars and application forms can be obtained from: The Personnel Department, AFRC Institute of Food Research, Shinfield, Reading, Berkshire RG2 9AT, Tel (0734) 883103 Ext 2299, quoting the appropriate reference numbers.

Closing date for the receipt of completed applications: 16th August 1990.

The AFRC is an equal opportunities employer.

(4327)A

UNIVERSITY OF DUBLIN

TRINITY COLLEGE

TEMPORARY LECTURER IN GENETICS

Applications are invited for the above post in the Department of Genetics at Trinity College, Dublin, tenable for a period of nine months commencing 1st October, 1990.

Candidates should hold a Higher degree; preference will be given to applicants who have a special interest in *either* molecular genetics *or* population and quantitative genetics.

Salary Scale: IR£11,857-IR£25,421 per annum

Appointment will be made within the salary range IR£11,857-IR£16,702 per annum, at a point commensurate with qualifications and experience to date.

Application forms and further particulars relating to this post may be obtained from:

Establishment Officer
Staff Office
Trinity College
Dublin 2
Tel: 772941 ext 1678
Fax 772169

The closing date for receipt of completed applications will be 31st August, 1990.

Trinity College is an Equal Opportunities Employer.

(4169)A

VICTORIA UNIVERSITY OF WELLINGTON
New Zealand

LIMITED TERM LECTURESHIP IN GEOLOGY

Applications are invited for a three year appointment available from 1st February 1991. Whilst the appointment may be in any field of geology, applications from those active in petrology, geochemistry or structural geology research may be preferred. The appointee will be expected to teach in one of these fields and may be required to contribute to other courses.

The salary scale for Lecturers is NZ$36,000-NZ$47,200 per annum.

Conditions of appointment and method of application are available from Appointments (38232), Association of Commonwealth Universities, 36 Gordon Square, London WC1H 0PF, or from the Appointments Administrator, Victoria University of Wellington, Po Box 600, Wellington, New Zealand (fax 04-711 700), with whom applications close on 15th September, 1990.

The University is an equal opportunity employer. (W7598)A

DIRECTOR, BIOLOGICAL RESEARCH & DEVELOPMENT

Lead a team of scientists in large scope projects.

Wyeth-Ayerst Research is one of the world leaders in pharmaceutical research and development. Currently we seek a top level individual to join our fast paced, innovative global research environment as Director of Biological Research and Development.

Responsibilities will include overall supervision of our Biological Pilot Plant, planning the direction and scope of research and development with Assistant VP, as well as providing expertise and solutions to clinical R&D and production staff involved in product development. We are looking for an individual with a successful track record in biological research and development who is recognized internally and externally as a highly qualified resource in infectious diseases and vaccine development. You will have the opportunity for publication and assure publication of scientific accomplishments for department.

Ideal candidate will have an M.D. or Ph.D. degree in biological sciences, and a minimum of 9 years experience in the administration and performance of biological research. A strong background in infectious diseases, molecular biology, virology, immunology and tissue culture is required, the ability to stay current with new developments in the field is essential.

Explore your exceptional career advantages with Wyeth-Ayerst. We offer a competitive salary, comprehensive company benefits and an innovative environment with top professional colleagues. For confidential consideration, forward your resume with salary requirements to: **Wyeth-Ayerst, Research Personnel, Position #112, P.O. Box 8299, Philadelphia, PA 19101.** Equal opportunity employer, M/F/H/V responses encouraged. (NW5596)A

WYETH
AYERST
TM

Nestlé

LINOR, the largest NESTLE Food Products Development Center is situated in Switzerland at Orbe, about 20 miles north of Lausanne.

You have

- A top class technical background in food cereal technology
- An understanding of the influence of raw materials on final product quality
- An industrial experience

You have

- A creative approach to problems
- An experience of applying chemical engineering principles to solving practical problems in the food industry
- An expertise in computer modeling or simulation

We can offer you a new dimension in your professional activities as project leader in

CEREAL

Your tasks will be:

- The creation of new products of dietetic and infant cereals
- The development of new technologies for the NESTLE group

COFFEE

Your main activities will be:

- A critical analysis of our operations at a pilot level
- The processes and products development for all NESTLE companies throughout the world

If you have a university degree, a basic knowledge of French and good communication with people from different backgrounds, please write with career details to:

NESTEC LTD
LINOR – Food Products Development Center
CH 1350 Orbe
Switzerland

(W7597)A

RESEARCH SCIENTIST

(Molecular Genetics)

$A33,445-$A40,916 pa

CSIRO DIVISION OF ANIMAL HEALTH,
GLEBE, NSW, AUSTRALIA

THE PROJECT: The CSIRO Division of Animal Health has a major commitment to the development of vaccines for the control of bacterial, viral and parasitic diseases and delivery systems for maximisation of vaccine performance. The research team has received financial support from the Australian Wool Corporation for the development and evaluation of gram-negative enteric mutant organisms as delivery vehicles for foreign antigens, particularly those of helminth parasites of sheep.

THE APPOINTEE: The appointee will undertake cloning and develop/evaluate expression systems, particularly for parasite antigens in gram-negative enteric mutant organisms (**Escherichia coli** and **Salmonella** species). The appointee will also be expected to participate in the immunological evaluation of the expressed antigens.

QUALIFICATIONS: The applicant should be self-motivated and have completed or almost completed a Ph.D degree or equivalent in molecular genetics. Proven research and organisational skills within this field are essential.

TENURE: Indefinite with Australian Government Superannuation benefits.

MORE INFORMATION: Prospective applicants are invited to telephone Dr T. K. Mukkur (02) 660 4411 or fax (02) 692 8561 for detailed information. A copy of the duty statement and selection criteria can be obtained from Ms M. Hunter.

APPLICATIONS: Applications with full curriculum vitae, the names of at least two professional referees and quoting reference number MGL23 should be forwarded to:

The Head of Laboratory,
Private Bag No. 1
P.O., Glebe, 2037
NSW Australia

no later than the 31st August 1990. (W7609)A

CSIRO
AUSTRALIA

CSIRO IS AN EQUAL OPPORTUNITY EMPLOYER

NATIONAL UNIVERSITY OF SINGAPORE

Department of Medicine

Applications are invited for appointment as

CLINICAL BIOCHEMIST

in the Department of Medicine. Candidates should preferably have a Masters or PhD degree in Biology or Biochemistry. those with a relevant Honours degree may also be considered. Experience in molecular biology would also be useful. The following are research projects that the Department is undertaking:

1. Natural history and serology of patients with chronic hepatitis B infection
2. Vaccination studies in hepatitis B
3. Interferon treatment of chronic hepatitis B
4. Interferon production in chronic hepatitis B infection
5. Serology of family members of hepatitis B carriers
6. The role of aflatoxins in hepatocellular carcinoma
7. Hepatitis C studies
8. Basic research on liver function

Specific duties and responsibilites include the following:

(a) daily supervision of laboratory staff
(b) responsible for stock taking, maintenance and purchasing of equipment and supplies
(c) administration of Hepatology Research Laboratory
(d) help in preparing protocols for research projects ie technical aspects
(e) quality control of all results done in the laboratory

The successful candidate may be appointed up to three years at a time.

Gross annual emoluments range from S$22,090 to S$38,650/S$57,300 (STG£1 = S$3.24 approx.). The commencing salary is dependent upon the qualifications and experience of the appointee.

Leave and medical benefits will be provided. Depending on the type of contract offered, other benefits may include: provident fund benefits or an end-of-contract gratuity, a settling-in allowance of S$1,000 or S$2,000, subsidised housing at nominal rentals ranging from S$100 to S$216 p.m., education allowance for up to three children subject to a maximum of S$10,000 per annum per child, passage assistance and baggage allowance for the transportation of personal effects to Singapore.

Candidates should write to the **Director, Personnel Department, National University of Singapore, 10 Kent Ridge Crescent, Singapore 0511,** giving their curriculum vitae and the names and addresses of three referees who can comment on their research ability and/or professional competence.

Enquiries may also be sent through BITNET to: **PERLT @ NUS3090,** or through **Telefax: (65) 7783948.** (W7614)A

Leicester University

Department of Anatomy

RESEARCH ASSISTANT

Applications are invited for the post of Research Assistant Grade IB to work on a three year project studying arterial chemoreceptors in the new born. The work involves physiological and biochemical techniques and would suit a recent graduate holding at least an Upper Second Class Honours degree in either of these disciplines. Salary on range RA1B (£11,399 — £13,495 p.a.).

Written applications should be sent to **Dr D.J. Pallot, Department of Anatomy, University of Leicester, PO Box 138, University Road, Leicester LE1 9HN** enclosing a full curriculum vitae and the names and addresses of two academic referees.

Closing date for applications: 31st August, 1990. (4329)A

UNIVERSITY OF SOUTHAMPTON

Department of Physiology and Pharmacology

LECTURESHIP

Applications are invited for a Lectureship (up to 3 years) which has been created by the award of a Wellcome Trust Senior Fellowship to Dr Howard Wheal. The successful candidate will be expected to contribute to the undergraduate and postgraduate teaching programmes in neurophysiology and conduct research complementary to that of our existing Neurophysiology group. Excellent facilities are available and collaboration will be actively encouraged.

Salary on Lecturer Grade A (up to £16,775 per annum [scale to be approved] will be dependent on qualifications and experience. Initial enquiries should be directed to Dr Howard Wheal (Tel: 0703 594275).

Please quote reference number: M/442.

POST DOCTORAL RESEARCH POSITIONS

Applications are also requested from suitably qualified candidates to join our research into CNS function. The major research areas as the properties of neurones and synaptic function in the hippocampus and in a model of epilepsy (HW); and the modulation and distribution of Ca ion channels (JC). The techniques currently in use are: conventional electrode recordings from tissue slices, patch-clamping and optical measurements including confocal microscopy. Research is currently funded by the MRC, SERC, Wellcome Trust and Wessex Medical School Trust.

Salary, (Grade 1A, up to £12,086 [scale to be approved] will be dependent on qualifications and experience. Initial enquiries should be directed to Dr John Chad (Tel: 0703 594292 - Fax: 0703 594319) or Dr Howard Wheal (Tel: 0703 594275).

Please quote reference number: M/443.

Applications (5 copies), including C.V. and the names and addresses of 3 referees, should be sent to **the Staffing Department (Ref: M/442 or M/443), University of Southampton, Highfield, Southampton, SO9 5NH. (Tel: 0703 592398),** from whom further particulars may also be obtained.

Closing date: 1st September, 1990.

"Working for Equal Opportunities". (4314)A

POSTDOCTORAL POSITION

Position available for an individual with a Ph.D. and experience in molecular biology. The successful applicant will be engaged in research on the biochemical and molecular mechanisms by which angiotensin peptides and other hormonal and pharmacological stimuli mediate cardiac muscle cell growth. There will also be the opportunity to engage in research defining the role of a localized intracardiac reninangiotensin system in mediating cardiac muscle cell growth, with an emphasis on the regulation of the precursor genes for this system. Competitive salary and fringe benefits are available.

Please send CV and three names or letters of reference to: **Dr. Kenneth M. Baker, Weis Center for Research, Geisinger Clinic, Danville, PA 17822.**

Equal opportunity employer.

(NW5558)A

POSTDOCTORAL POSITIONS

PROTEIN ENGINEERING NETWORK CENTRE OF EXCELLENCE PROGRAM

Challenging postdoctoral positions are available in the general area of the de novo design and synthesis of novel peptides and proteins with desired structure and biological activity. Background in protein chemistry and spectroscopy is desired.

Starting salary $25,000 and is available for 4 years, beginning immediately.

Send detailed CV and request 3 letters of reference to be directed to: **Dr C M Kay, Department of Biochemistry, University of Alberta, Edmonton, Alberta T6G 2H7**

The University of Alberta is committed to the principle of equity in employment.

(NW5579)A

LABORATORY DIRECTOR

Bio Ventures Division – Worcester

The Bio Ventures Division of Failure Analysis Associates, Inc., an international engineering consulting firm, currently seeks a Laboratory Director with outstanding academic and professional credentials.

You will manage an expanding, state-of-the-art cell culture laboratory, determine project goals and provide project technical leadership. You will also ensure that projects are clearly defined and communicate project status and results. Establishing applicable theoretical bases and principles, manpower and expense requirements, and methodologies to be followed will also be among your responsibilities. You need a PhD and 3-5 years' post-doctoral experience in mammalian cell structure, cellular physiology, biology of cell-to-cell interaction, or mammalian hematopoietic stem cell cultures. Experience in molecular biology is helpful. The successful candidate will be a motivated self-starter with excellent communication skills. You must be able to effectively manage and interact with staff.

We offer a comprehensive compensation and benefits package. For confidential consideration, please send your resume to, Vernetta Wilson, AD# NSM854, Human Resources Dept., Failure Analysis Associates, Inc., P.O. Box 3015, Menlo Park, CA 94025, or fax to (415) 328-3049. EOE

(NW5598)A

Failure Analysis Associates.

THE UNIVERSITY OF AUCKLAND
New Zealand

HIGH FIELD NMR POSITION

A vacancy exists in the University of Auckland, Department of Chemistry for a Senior Technical Officer, to service the High Field NMR spectrometer.

Applicants should have a PhD or equivalent research experience in NMR spectroscopy including experience with multidimensional NMR modern pulse sequences and their implementation, computer hardware and software and, preferably, some knowledge of electronics, along with evidence of organisational ability.

The successful applicant will be responsible to the Head of the Department through the High Field NMR committee for management and operation of a Bruker AM400 spectrometer, associated facilities and other NMR spectrometers as they are acquired.

Duties will include: Overseeing the day-to-day operation of the instrument and advising staff and students; undertaking routine maintenance; up-dating of software; implementing new NMR techniques e.g. pulse sequences; training of operators; administration of associated accounting; maintaining data distribution; participation in collaborative research projects.

Salary up to NZ$40,000 per annum depending on qualifications and experience.

The initial term of appointment will be for one year, renewable thereafter by agreement. Consideration may be given with regard to relocation costs.

Further information from Dr Jan Coddington, tel. 64-9-737999 ext. 8951.

Copies of the Conditions of Appointment are available from the University Personnel Department, 24 Princes Street, Auckland, New Zealand (tel. 64-9-737999 ext. 7470 or 7676), or from Appointments (38246), Association of Commonwealth Universities, 36 Gordon Square, London WC1H 0PF.

Written applications, as per method of application contained in the conditions of Appointment close with the Registrar, University of Auckland, Private Bag, Auckland, New Zealand on 26th October, 1990. Please quote vacancy 90/50.

The University of Auckland is an equal opportunities employer. (W7608)A

SMH
ST. MICHAEL'S
HOSPITAL

Director
Research Institute

St Michael's Hospital, a major teaching hospital affiliated with the University of Toronto, has created a new Research Institute and seeks applicants for the post of Director.

The position requires an imaginative, creative and dynamic scientist with excellent leadership skills to:

☐ Provide scientific leadership for the Institute, establish new research programs and stimulate multidisciplinary collaboration.

☐ Build and lead an internationally recognized program in health research.

☐ Shape the future of the Institute through significant new recruitment of both fundamental and clinical scientists.

The Research Institute will include a new facility within St Michael's Hospital. It will accommodate ongoing research programs. Excellent opportunities exist for interacting with a variety of strong research programs throughout the hospital.

In accordance with Canadian immigration requirements, priority will be given to Canadian citizens and permanent residents.

Applicants should submit a curriculum vitae and the names and addresses of three references by November 30th, 1990 to: Chairman, Search Committee for Research Institute Director, Administration, St Michael's Hospital, 30 Bond Street, Toronto, Ontario M5B 1W8. Fax: (416) 864-5352.

St Michael's Hospital is a major Canadian university teaching hospital. St Michael's is committed to studying the causes, treatment and prevention of disease.

(NW5580)A

Liverpool School of Tropical Medicine

DIRECTOR OF THE SCHOOL

The Council of the Liverpool School of Tropical Medicine invites applications for the new post of Director of the School.

The School, a registered charity affiliated to the University of Liverpool, is one of the few postgraduate centres of excellence in the world in the field of tropical medicine. Its principal functions are research, teaching and consultative activities. The School is extensively involved in national and international programmes to control tropical and infectious diseases and to develop effective health care systems.

The new post of Director has arisen from the Report of an Academic Review Working Party which recommended changes in the School's organization and management, including the integration of the School's six Departments into a single Department. The Council is therefore looking for a Director with substantial managerial skills as well as a person who will provide strong and able academic leadership.

The Director will be the Chief Executive Officer and Academic Head of the School and should have international standing appropriate to the School's reputation, and professional or research interests relevant to the School's work. The Director will play an active role in promoting the School nationally and internationally and in seeking sources of funding,and must have wide knowledge and experience of international agencies, and governmental and non-governmental organizations in the health sector. The salary is expected to be £45,000 - £50,000 p.a. The post will carry Professorial title and status in the University of Liverpool.

Closing date for applications is 30 September, 1990.

Applications, which should contain a statement of the candidate's age, experience, qualifications and published work, together with the names and addresses of three referees, should be sent to the Chairman of Council, Liverpool School of Tropical Medicine, Pembroke Place, Liverpool, L3 5QA, from whom further particulars may be obtained

(4325)A

The School is an Equal Opportunity Employer

The Department of Molecular Biology at Princeton University

invites applications for a

junior level position from investigators who use the mouse as an experimental system. Areas of specialization may include (but are not restricted to) immunology, genetics, neurobiology, developmental biology, chromosome structure, or genome analysis. Candidates should have a Ph.D degree and appropriate experience in mouse molecular biology. Applicants should send a curriculum vitae, a summary of research interests, and the names of at least three referees to:

Mouse Molecular Biology Search Committee
Department of Molecular Biology
Princeton University
Princeton, New Jersey 08544

Application deadline is 20th October, 1990.

Princeton University is an Equal Opportunity/Affirmative Action Employer. (NW5564)A

THE UNIVERSITY OF BRITISH COLUMBIA

ANATOMIST — TENURE TRACK POSITION

Assistant Professor Level

PhD, MD or equivalent doctoral training with at least two years' of post-doctoral experience in research teaching. Appointees should have independent research interests and will be expected to qualify for research support from extra-department sources. Salary is from a university-based departmental budget and is dependent on qualifications and experience.

Applicants should be qualified to assist in the teaching of any of the branches of Anatomy to medical and dental students. An involvement in graduate teaching is also expected in the appointee's field of academic interest. Starting date: January 1st, 1991 or July 1st, 1991.

Apply before September 30th, 1990, to the following: Dr Charles E Slonecker, Department of Anatomy, 2177 Wesbrook Mall, The University of British Columbia, Vancouver, BC, Canada V6T 1W5. Applications should be accompanied by a curriculum vitae and the names of three academic referees.

In accordance with Canadian immigration requirements, priority will be given to Canadian citizens and permanent residents of Canada. The University of British Columbia is committed to the Federal Government's employment equity program and encourages applications from all qualified individuals.

(NW5578)A

University College of Swansea

SENIOR RESEARCH ASSISTANT

Applications are invited for th vacancy of Senior Researc Assistant in the School of Biol ogical Sciences to work on a SERC Collaborative project be tween the University Colleg of Swansea and UMDS o Guy's and St Thomas' Hospi tals, which is based mainly i Swansea. The successful ap plicant will undertake a stud on the role of lipoxins, a nove class of eicosanoid, in inflam mation and immune regulatio using fish as a model syste and will join a group looking a various aspects of eicosanoi biosynthesis and functioning.

The appointment, which wi commence from the sooes date that can be arranged, wi be renewable on an annua basis for up to three years and the commencing salary will be £10,458 per annum togethe with USS benefits, if required.

Further particulars and application forms (2 copies) may be obtained from the Personnel Office, University College of Swansea, Singleton Park, Swansea SA2 8PP, to which office they should be returned by Friday August 17, 1990.

(4311)A

University of Southampton

Department of Biochemistry

SERC-funded Post-Doctoral Research Assistant to work with Professor C Anthony on "Structures and interactions of the quinoprotein methanol dehydrogenase and cytochrome c". Applicants should preferably have experience in development of bacterial protein purification and modification methods.

The post is for two years; salary within range £11,139-£13,495 per annum (scale to be approved).

Applications (2 copies) should be sent with names of two referees, by 31st August 1990, to the Staffing Department (Ref: M/460), University of Southampton, Highfield, Southampton SO9 5NH. Working for equal opportunities. (4337)A

RESEARCH SCIENTIST — COMPLEX CARBOHYDRATES

Glycomed, which emphasizes the development of carbohydrates for pharmaceutical application, has an opening for a biochemist, who must have at least two years' experience in carbohydrate research in the area set forth below, in either the graduate academic or the industrial level. Minimum educational requirement is a PhD in biochemistry or cell biology. Primary duties will include research in isolation and structural analysis of complex carbohydrates, specifically glycosaminoglycans, in animal cell culture techniques and in studies of carbohydrate metabolism in whole cells and cell extracts. Further duties will include research in immunochemistry and in isolation and characterization of cell mutants. The salary range for this position is $45,000-$46,000, depending upon qualifications.

Glycomed, located in the San Francisco Bay Area, offers a full range of employee benefits, including stock options. Resumes must include detailed descriptions to document experience in the research areas required for this position and must be postmarked no later than 8/31/90, in order to be considered. Candidates must be able to show that they have the legal right to work in the United States.

Please send resumes and three detailed written references to: Manager of Human Resources, Glycomed, 860 Atlantic Avenue, Alameda, CA 94501.

AN EQUAL OPPORTUNITY EMPLOYER

(NW5592)A

Cancer Research Campaign

- We are the biggest funder of cancer research scientists, supporting some 700 projects in universities, medical schools and research institutes throughout Britain
- We have developed a strong interface between research and clinical therapy, facilitating the effective development and implementation of cancer treatments
- Of the nearly £40 million we raised through voluntary contributions in 1989, 92% went directly into research - accounting for around 40% of all cancer research in the UK.

CRC LABORATORIES, DEPT OF BIOCHEMISTRY, UNIVERSITY OF DUNDEE,

A number of positions are available for enthusiastic and energetic people to join the Cell Transformation Group and the Cell Structure Group in the new CRC Laboratories at Dundee University. These two established groups will be moving to Scotland later this year to form part of a new Cancer Research Campaign initiative at the University of Dundee. The CRC Laboratories at Dundee are being furnished and equipped to very high technological standards within the University's prestigious Biochemistry Department, which is one of the most highly funded in Britain. The University of Dundee is 1 hour from Edinburgh airport with its hourly shuttle flights to London. Housing and accommodation costs in Dundee are among the lowest in the UK and access to outdoor recreational facilities is unrivalled.

POST DOCTORAL SCIENTIST AND RESEARCH ASSISTANT

Two new Research Assistantship posts at graduate or postdoctoral levels are available with the Cell Structure Group (Dr E B Lane) to work on the structure and function of cytoskeletal intermediate filaments in differentiation and tumourogenesis. The project/s will involve extensive use of monoclonal antibodies, molecular biology and high-resolution microscopy. Salaries within the ranges £11,399-£13,495 (graduate) or £11,399-£18,165 (postdoctoral).

RESEARCH ASSISTANTS

Two new Research Assistantship posts (technical, SO/SSO) are available within the Cell Transformation Group (Prof D P Lane) to work on p53 and retinoblastoma tumour suppressor genes. Experience in modern molecular biology and molecular immunochemistry is desirable. Salaries dependant on age and experience.

We also wish to attract both a SECRETARY/PA and LABORATORY MANAGER to work between the two groups. These are both key positions in which you will make valuable contributions to the success and achievements of these teams, therefore relevant experience is highly desirable.

Well-qualified applicants are encouraged to contact David or Birgit Lane as soon as possible through the Biochemistry Department, University of Dundee, Dundee DD1 4HN, Scotland or at their current address at the ICRF Clare Hall Laboratories, South Mimms, Potters Bar, Hertfordshire EN6 3LD, enclosing full curriculum vitae and the names of three referees. The appointments can be made as soon as good candidates have been found. Applicants are advised that smoking is prohibited within these laboratories.

CRC BEATSON LABORATORIES, THE BEATSON INSTITUTE FOR CANCER RESEARCH, GLASGOW

POST DOCTORAL SCIENTIST

A scientist position for up to five years is available in Dr Paul Harrison's group which currently has two main interests : the regulation of seleno-protein gene expression and the anti-carcinogenic effects of selenium; and changes in gene expression associated with tumour progression in leukaemias. The appointment will be for three or five years in the first instance within the salary range £11,399 to £24,930 p.a. depending on age and experience, with the possibility of extension thereafter depending on progress and proven ability. Further information can be obtained from Dr P R Harrison (The Beatson Institute for Cancer Research, Garscube Estate, Switchback Road, Bearsden, Glasgow, G61 1BD. Tel: 041-942 9361) to whom applications with C.V. and the names of two referees should be sent. (4328)A

Fighting cancer on all fronts.

UNIVERSITY OF YORK

DEPARTMENT OF CHEMISTRY

LECTURESHIP IN PROTEIN CRYSTALLOGRAPHY

Applications are invited for a Lectureship in Physical Chemistry with special research interests in protein crystallography.

It is expected that research will be carried out in our existing laboratories which have excellent X-ray facilities, computational and graphics devices and supporting molecular biology. The Department of Chemistry wishes to establish a commitment to small molecular crystallography, and the candidates will be responsible for management of this programme.

Teaching will be in the area of physical chemistry with opportunities to teach biological and structural chemistry.

Salary will be within Lecturer Grade A (£12,086-£16,755 per annum).

Six copies of applications with full curriculum vitae and naming three referees, should be sent by 5 September 1990 to the Personnel Office, University of York, Heslington, York YO1 5DD from whom further details are available. Please quote reference number 6/3302. (4309)A

UNIVERSITY OF READING

Applications are invited for the post of

EXPERIMENTAL ASSISTANT

in the Department of Agriculture to take part in a programme aimed at obtaining twin calvings in beef cows by embryo transplantation. Experience of calving is essential. The initial appointment will be for three years on the salary scale £6,789 to £9,257 pa. Overtime will be paid. Rent-free accommodation will be available towards the end of the year.

Application forms available from the Personnel Office, University of Reading, Whiteknights, PO Box 217, Reading, RG6 2AH. Telephone (0734) 318751. Please quote reference T31. (4307)A

THE UNIVERSITY OF BRITISH COLUMBIA

DEPARTMENT OF CHEMISTRY

ASSISTANT PROFESSORSHIP

The Departments of Chemistry & Biochemistry, University of British Columbia, invite applications for a tenure-track assistant professorship in Protein NMR, starting July 1, 1991. Preference will be given to candidates who propose to carry out research in the determination of 3-dimensional structures of proteins by means of NMR. A Ph.D., postdoctoral experience, and a strong research track record are required. The successful candidate would be expected to teach chemistry and/or biochemistry courses at the undergraduate and graduate level. Excellent satrt-up funding is available, including the provision of funds for a 500 MHz NMR instrument. The salary will be commensurate with education and experience.

The University of British Columbia is committed to the Federal Government's employment equity program and encourages applications from all qualified individuals. The position is subject to final budgetary approval and, in accordance with Canadian immigration requirements, priority will be given to Canadian citizens and permanent residents.

Applications consisting of a curriculum vitae, list of publications, summary of research interests, a detailed research proposal, and the names and addresses of at least three references should be sent to: **Professor S Withers, Chairperson, Search Committee, Department of Chemistry, University of British Columbia, 2036 Main Mall, Vancouver, British Columbia, Canada V6T 1Y6.**

Closing date for applications will be 1st October, 1990. (NW5577)A

THE UNIVERSITY
OF BIRMINGHAM

SCHOOL OF BIOCHEMISTRY

POSTDOCTORAL FELLOW

Applications are invited from molecular biologists to work on a project with Professor Alan Colman investigating protein secretion during development of the frog, Xenopus laevis. Project involves cloning of maternal mRNAs which encode membrane or secretory proteins. Using reagents generated from these clones, a search will be undertaken for encoded products whose surface presentation during early development is regulated both in time and space, thereby allowing a test of our premise that such regulation has a determinative role during early development.

Informal enquiries to Professor A Colman 021-414 5408.

Salary on the range £11,399-£18,165.

Post is funded by the Wellcome Trust from 1st November 1990 for 4 years. Ref: S13001.

POSTDOCTORAL RESEARCH FELLOW

'The enzymology and molecular genetics of the formation of the isoprenoid monomer, isopentenyl diphosphate in higher plants'

This post (tenable for 3 years from 1st October 1990) involves use of modern protein separation techniques to improve purification procedures already developed in this laboratory, immunocytochemical techniques of electron microscopy and identification of genes in already cloned cDNA libraries.

Informal enquiries to Dr R G O Kekwick 021-414 5416.

Salary on the range £11,399-£18,165. Ref: S13083

POSTDOCTORAL RESEARCH FELLOW OR GRADUATE RESEARCH ASSOCIATE

Oxidation of Cysteine Derivatives: molecular basis and clinical correlations.

This post (tenable for 2 years from 1st October 1990) is funded by the MRC for work on enzyme systems controlling the S-oxidation of cysteine derivatives in man. Reduced individual capacity for this reaction is closely correlated with chronic liver disease, and the molecular basis for this defect will be studied. Protein purification will be followed by construction and screening of cDNA libraries, in close collaboration with clinical colleagues at Queen Elizabeth Hospital, Birmingham.

Experience or interest in techniques of protein purification or molecular biology preferable.

Informal enquiries to Dr R H Waring 021-414 5421.

Salary on the range £11,399-£13,495. Ref: S1975

Application forms (returnable by 24th August 1990) and further particulars available from the Director of Staffing Services, The University of Birmingham, Edgbaston, Birmingham B15 2TT. Tel: 021 414 6483 (24 hours). Please quote appropriate reference.

The University is an equal opportunities employer.

(4323)A

UNIVERSITY OF EDINBURGH
Department of Veterinary Pathology

TWO LECTURESHIPS

Applications are invited for the following new posts

1. Lectureship in Veterinary Pathology (Ref 1540A

Applicants for this post must be graduates in Veterinary Medicine since the person appointed will be expected to contribute to diagnostic veterinary pathology.

1. Lectureship in Veterinary Bacteriology (Ref 1540B

Although it is expected that the successful candidate for this post wil hold a veterinary degree, applications will also be considered from non-veterinary graduates with expertise/interest in veterinary bacteriology.

The individuals appointed to these posts will be joining a Department with considerable expertise in immunology and molecular pathology, and with major interests in the molecular basis of disease processes. The successful candidates will engage in the research, teaching and diagnostic work in Pathology and Bacteriology and be expected to develop a research programme in the paraclinical sciences.

The posts which are primarily intended for young individuals are on the Lecturer A scale (£12,086-£16,026).

Further particulars may be obtained from Professor I McConnell, Department of Veterinary Pathology, University of Edinburgh, Summerhall, Edinburgh EH9 1QH (Tel: 031-667 1011 ext 5285 or Fax: 031-662 0373) with whom applications, including curriculum vitae and names of three academic referees, should be lodged by 7th September, 1990 at the latest. Overseas applicants should register interest by fax and send one copy of their application. The positions are available now. When applying please quote the relevant reference number.

(4292)A

ST. GEORGE'S HOSPITAL MEDICAL SCHOOL

(University of London)

POSTDOCTORAL RESEARCH FELLOW (1a) or RESEARCH ASSISTANT (1b)

Department of Cellular and Molecular Sciences

Division of Haematology

Applications are invited for candidates to work on haemopoietic growth factor production in normal individuals and patients with aplastic anaemia and leukaemia. Candidates will be considered at a post-doctoral level, or as a graduate research assistant with a relevant honours degree.

Experience in recombinant DNA and/or tissue culture would be an advantage but is not essential as training will be given. Salary on the 1a scale for the Postdoctoral Research Fellow will be in the range £12,225-£14,148 per annum including London Allowance or on the 1b scale for the Research Assistant will be in the range £11,583-£14,148 per annum including London Allowance (1990 pay award pending). The post is for 3 years and is funded by the Marrow Environment Fund.

For an informal discussion or an informal visit to the Department contact: Dr Tim Rutherford (ext. 55487) or Dr Fran Gibson (ext. 55481) on 081-672 9944.

Further details and an application form from the Personnel Officer, St George's Hospital Medical School, Cranmer Terrace, London SW17 0RE. Tel: 081-784 2791 (24-hr answerphone). Please quote reference 91/90. Closing date 24th August, 1990.

(4303)A

KING'S COLLEGE SCHOOL OF MEDICINE AND DENTISTRY
of King's College London

MOLECULAR MEDICINE UNIT

We are looking for a

Personal Assistant to the Director

of our recently established Unit of Molecular Medicine. Responsibilities will include the development and provision of a number of central molecular biology services and will therefore involve both laboratory based work and a number of administrative duties. Previous experience in cell or molecular biology will be an advantage but not essential as training will be provided. The key qualities for this position, which will offer excellent opportunities for the development of career in molecular biology, are: a degree in a biological subject at the graduate or postgraduate level, reliability and drive.

Prospective applicants may obtain further details by contacting Dr Farzin Farzaneh, Director of the Unit on 071-326 3126.

This appointment, for three years in the first instance and renewable thereafter, will be on a Medical Technical Officer Grade 3 salary scale up to £15,256 (inclusive).

Applications in the form of a full curriculum vitae and supporting letter should be addressed to the Secretary of the School, King's College School of Medicine and Dentistry, Bessemer Road, London SE5 9 PJ to arrive by 24th August, 1990.

(4306)A

RESEARCH SCIENTIST/ SENIOR RESEARCH SCIENTIST

$A32,471-$A47,070

DIVISION OF WOOL TECHNOLOGY
RYDE, N.S.W. AUSTRALIA

General: Research at the CSIRO Division of Wool Technology is producing important innovations in wool marketing, processing, and product development. The aim of the Division, which has a staff of more than 400 is to increase worldwide demand for Australian wool.

The Division has been responsible for the introduction and implementation of objective measurement of the raw wool clip, and is in the process of introducing objective measurement to the fabric and garment industries.

The Position: A Research Scientist is required to establish quantitative relationships between raw wool properties, processing performance and fabric properties. This work is to be performed in our mini-processing mill, in conjunction with industry.

The Person: After familiarising yourself with the appropriate physical mechanisms involved, you will be expected to lead an experimental program with the view to demonstrating the relationship between wool properties and fabric properties.

You will be an innovative, pragmatic physicist with a good knowledge of mechanics and an aptitude for mechanical processing. Your interest will be to apply physics to problems in the wool industry.

Conditions: Indefinite with Australian Government Superannuation benefits.

Qualifications: A PhD degree in a physical science or equivalent.

More Information: Prospective applicants are encouraged to obtain a detailed job description and selection criteria from Mrs K. Murie on (02) 809 9379 and further information concerning the position from Dr S. deJong on (02) 809 9444.

Applications: Applications should relate to the selection criteria and should provide details of relevant personal particulars including qualifications and experience and the names of at least two referees.

Applications quoting Position No. C8068 should reach the following address by 31st August 1990:

**The Assistant Chief,
CSIRO Division of Wool
Technology
PO Box 7
RYDE NSW 2112 AUSTRALIA** (W7602)A

CSIRO
AUSTRALIA

CSIRO IS AN EQUAL OPPORTUNITY EMPLOYER
CSIRO PROMOTES A SMOKE-FREE ENVIRONMENT

POSTDOCTORAL POSITION
Molecular Endocrinology

NIH-funded program on autocrine regulation of parathyroid secretion by chromogranin peptides. Research utilizes cell biology, protein chemistry, molecular biology. Exceptional research environment in a major medical/dental health science center. Faculty appointment possible for qualified candidate. Send resume, 3 references to Dr David V. Cohn, Chair, Department of Oral Health, School of Dentistry, University of Louisville, Louisville, KY 40292. Tel. (502) 588-7507.

Affirmative Action/Equal Opportunity Employer. (NW5587)A

POSTDOCTORAL ASSOCIATE

to study regulation of eye growth (especially of scleral chondrocytes) by retinal factors during development. Training in cell or developmental biology and experience in tissue culture would be useful. For background on the research problem see Nature 345:477 (June 7, 1990) and Science, 237:76 (1987).

Josh Wallman, Dept of Biology, City College, City University of New York, New York, NY 10031. (NW5586)A

EMBL

The European Molecular Biology Laboratory, an international research organization situated in Heidelberg, West Germany, has vacancies for:

GROUP LEADERS in NMR of MACROMOLECULES

EMBL is expanding its activities in structural biology into the field of structure determination by NMR. The Biological Structures and Biocomputing Programme is seeking candidates for two Group Leader positions from scientists with experience in computational and/or experimental aspects of NMR as applied to the solution of three-dimensional structures of biological macromolecules. The successful applicants will develop their own research programmes and also undertake research in collaboration with other scientists at EMBL.

Applicants should have a PhD and relevant post-doctoral experience.

Candidates will be invited to visit the Laboratory in Heidelberg in the coming weeks for discussions and to give a seminar. Please send a C.V., a brief research proposal, and a list of relevant publications, together with dates when it would be suitable for you to visit EMBL, your telephone and Fax numbers to the Personnel Section.

Applicants should contact Dr. S. Fuller or Dr. D. Suck, Biological Structures and Biocomputing Programme for details.

Closing date for applications: 30.09.1990

Please quote ref. no. 90/30 (W7607)A

*EMBL
Personnel Section,
Postfach 10.2209
D-6900 Heidelberg
Federal Republic
of Germany*

THE QUEEN'S UNIVERSITY OF BELFAST
RESEARCH FELLOW/ASSOCIATE
Department of Ophthalmology

The above post is available for one year in the first instance, with the possibility of extension to five years, to study cell interactions between neural, glial and vascular cells. Applicants must have an Honours Degree in Cell Biology/Biochemistry or a related discipline and should preferably have a PhD or an MSc and several years relevant research experience. Experience in molecular biology and tissue culture techniques and knowledge of the field of growth factors and regulatory peptides would be an advantage.

Salary scale: £11,399-£13,495 per annum, placing depending on qualifications and experience.

Applicants, quoting ref. 90/00, should submit a curriculum vitae, including the names and addresses of two referees to the Personnel Officer, The Queen's University of Belfast BT7 1NN, Northern Ireland. Closing date: 24th August, 1990.

The University is an Equal Opportunity Employer. The University may decide to interview only those applicants who appear, from the information available, to be the most suitable in terms of experience, qualifications and other requirements of the post. (4319)A

The Department of Molecular Biology at Princeton University

invites applications for a

tenure track position at the Assistant Professor level in the area of Developmental Neurobiology. We are especially interested in individuals who are working on the development or functioning of the nervous system in organisms which are amenable to both genetic and molecular analysis. Applicants should send a curriculum vitae, a summary of research interests, and the names of at least three referees to:

**Developmental Genetics and Neurobiology Search Committee
Department of Molecular Biology
Princeton University
Princeton, New Jersey 08544**

Application deadline is 20th October, 1990.

Princeton University is an Equal Opportunity/Affirmative Action Employer. (NW5563)A

University of Waikato

Te Whare Wānanga o Waikato

Hamilton, New Zealand

Applications are invited for the following appointments:

SENIOR LECTURER/LECTURER IN FORESTRY

The University has recently initiated a programme in Forestry that will lead to a BSc (Tech) or MSc (Tech) degree with a forestry major. A significant portion of the practical side of training will be conducted in Industry and at the Waiariki Polytechnic at Rotorua.

The University wishes to make an appointment in Forestry with emphasis on silviculture and forest production. The appointment will be made in the School of Science and Technology and the appointee will be in the staff of the Department of Biological Science. Initially the appointee will be required to teach in the second year course in Forestry and other related undergraduate courses, to play a major role in the administration of the BSc (Tech) programme and be active in Forest related research.

Applicants would be expected to have an advanced forestry degree and to have wide experience of the industry with emphasis on production forestry or agroforestry. As a significant part of the position will be concerned with liaison with the Forestry industry, with research institutions and with employers, experience in these areas would be an added benefit.

Enquiries of an academic nature may be made to Professor WB Silvester (tel (64) 71 562889, Fax (64) 71 560115). Applications quoting reference number A90/62 close on 31st August, 1990.

LECTURER IN EARTH SCIENCES

The University of Waikato has a vacancy for a Lectureship in the Department of Earth Sciences in the general area of soil mechanics, in which applicants should have a secondary interest in engineering geology or soil physics or another related field that supports the teaching and research programme of the Department. The appointee will assist with teaching, laboratory classes, and fieldwork in established Earth Science courses; will be required to supervise graduate and postgraduate research projects; and will foster and become involved in personal and/or collaborative pure and applied research programmes.

Applicants preferably should have a doctorate, or be about to gain one, or should have equivalent practical experience and research qualities in their field. The position is available from late 1990 but must be taken up by 1st February, 1991.

Further information about the activities and facilities of the Department can be obtained from Associate Professor C S Nelson, Chairperson of the Department of Earth Sciences (tel (64) 71 562889 Fax (64) 71 560115). Applications quoting reference number A90/56 close on 28th September, 1990.

The current salary scale for Lecturers is NZ$36,000-NZ$47,200 and for Senior Lecturers NZ$50,000-NZ$64,500.

Information on the conditions of appointment and method of application can be obtained from the Academic Staff Registrar, University of Waikato, Private Bag 3105, Hamilton, New Zealand. (Tel (064) 71 562889, Fax (064) 71 560135).

Places for appointees' children may be available in the creche run by the Campus Creche Society (Inc). The University welcomes applications from suitable people regardless of race, creed, marital status or disability. (W7611)A

MOLECULAR BIOLOGIST

The Department of Surgery, division of Surgical Oncology has a full time faculty position at the Assistant Professor level effective immediately. Candidates should have a PhD and at least three years of relevant postdoctoral experience. Research areas: all pertinent skills relating to molecular biology including DNA and RNA work; molecular immunology, antibody production and immunoassays; generating synthetic oligonucleotides and DNA sequencing; genomic and cDNA cloning; subtraction hybridization; protein chemistry including amino acid sequencing and HPLC. Should be able to work independently and must be motivated to develop an externally funded research program. Please forward curriculum vitae and references by October 1, 1990 to **T.S. Ravikumar, M.D., Yale University School of Medicine, Department of Surgery, P.O. Box 3333, New Haven, CT 06510.**

Yale University is an Affirmative Action Equal Opportunity Employer.

(NW5581)A

Leukaemia Research Fund

Experimental Haemopoiesis Unit
(Honorary Director: Professor Sir David Weatherall)
Institute of Molecular Medicine
University of Oxford

UNIT DIRECTOR

A unique opportunity to establish and develop a Unit to study the cellular and molecular regulation of haemopoiesis has been created by the Leukaemia Research Fund at the Institute of Molecular Medicine, John Radcliffe Hospital, Oxford.

Applicants should have a proven record of creative, original research combined with dynamic and innovative leadership. The successful candidate must demonstrate an ability to establish an international competitive programme in experimental haemopoiesis and to collaborate closely with other groups within the Institute. Salary will be commensurate with the importance of this position.

The Institute of Molecular Medicine is a recently formed multi-disciplinary development and houses a number of independent groups which are applying the techniques of molecular and cell biology to study human disease.

Further information can be obtained from the Honorary Director of the Unit, Professor Sir David Weatherall, Institute of Molecular Medicine, John Radcliffe Hospital, Oxford OX3 9DU to whom applications should be sent in writing with CV (5 copies) and the names and addresses of three referees. (4326)A

Closing date for applications: 14th September 1990.

UNIVERSITY OF BATH

Bath Institute for Rheumatic Diseases

MOLECULAR BIOLOGIST/CELL BIOLOGIST

A highly motivated post-doctoral research worker is required to join an interactive young team of 11 scientists working on the cellular and molecular biology of bone with particular interest in osteoporosis. Ongoing projects include the identification of novel and specific bone cell-associated antigens, the immortalisation of human bone cells and the localisation of osteotropic cytokines within human bone. We are particularly interested in hearing from candidates with molecular biology experience. A completed PhD is essential and post-doctoral experience preferred.

Excellent laboratory facilities are situated in the Institute for Rheumatic Diseases in the centre of Bath.

The post is available for 3 years in the first instance with the potential for renewal.

Salary on the University 1A scale £11,399 pa-£18,165 pa, starting point according to age and experience.

Applications, including a full curriculum vitae and the names, addresses and telephone numbers of two referees shoud be sent to the Personnel Officer, University of Bath, Claverton Down, Bath BA2 7AY quoting ref no: 90/170. Closing date: 24th August, 1990.

Informal enquiries can be directed to Dr Maxine Gowen. Tel: (0225) 448444. (4308)A

UNIVERSITY OF CAMBRIDGE

School of Clinical Medicine

RESEARCH ASSISTANT/ASSOCIATE

A position is available for either a graduate assistant or a postdoctoral fellow, for a 3 year project on the regulation of gene expression by thyroid hormone (PNAS [1990] 86: 9114-8). Experience with recombinant DNA or protein purification techniques would be an advantage.

The appointment will be on the Cambridge research workers scale up to £16,755 p.a., depending upon age and experience. Application with a full curriculum vitae and two referees should be sent to Dr VKK Chatterjee, Department of Medicine, Level 5, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ. Informal enquiries are welcome. Telephone: (0223) 336843, Fax: 0223 212920. (4295)A

UNIVERSITY OF SOUTHAMPTON

Trace Element Unit,
Clinical Biochemistry

TRACE ELEMENT RESEARCH IN CLINICAL AND ENVIRONMENTAL SCIENCE

Applications are invited from graduates with a good honours degree for the following appointments.

1. A three year appointment to investigate the bioavailability of aluminium from infant foods. *Please quote ref: M/458.*
2. A one year appointment, initially, to assess the apportionment to body lead of environmental sources using plasma-source mass spectrometry for stable lead isotope ratio measurements. *Please quote ref: M/459.*

For Post 1, applicants should have a first degree in nutrition or biochemistry; for Post 2 applicants should have a first degree in analytical/environmental science. Appointees will be encouraged to register for a higher degree.

Salary on University 1B Research Assistant scale: £11,399 to £13,395. (Scale to be approved).

Applications (2 copies) and requests for further information to Staffing Department (Ref: M/458 or M/459), University of Southampton, Highfield, Southampton, SO9 5NH (Tel: 0703 592398), as soon as possible.

Starting date: 1st September, 1990.

Working for equal opportunities. (4321)A

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of science

SULTAN QABOOS UNIVERSITY

COLLEGE OF SCIENCE

ADVERTISEMENT

Applications are invited for posts in the following specialisations:

FIRSTLY:

TEACHING STAFF:

EARTH SCIENCES DEPARTMENT:
Must have a proven ability and an experience in the fields related to Earth Sciences, specialists in Marine Geology, Sedimentology, Biostratigraphy, Remote Sensing, Basin analysis & Sedimentology; Applied and Environmental Geochemistry or Hydrogeology.

BIOLOGY DEPARTMENT:
Specialists in Genetics; Animal physiology, Behavioral Ecology and Conservation, Marine Biology and Oceanography.

CHEMISTRY DEPARTMENT: Specialists in Analytical Chemistry and Physical Chemistry.

PHYSICS DEPARTMENT: Specialists in surfaces and thin films.

MATHS AND COMPUTING DEPARTMENT: Computer Scientists.

SECONDLY:

DEPARTMENTAL SUPERINTENDENTS:
Applications are also invited for departmental superintendents for: Earth Sciences department, The Physics department, The Biology department and the Maths and Computing department to take charge of the laboratories and technical staff.

THIRDLY:

CHIEF TECHNICIAN:
To take charge of the large analytical instrument suite which includes Scanning Electron Microscope and Microanalyser, XRF & XRD.

BENEFITS:

Post	Monthly basic Salary	Annual Allowance
Professor	1770	80
Associate Professor	1330	60
Assistant Professor	1030	50
Departmental Superintendent	1030	50
Chief Technician	980	18
Salaries will be given in Omani Riyals. There is no income tax in the Sultanate. (1 Omani Riyal = 2.58 U.S. Dollars.)		

* In addition to that there is a 15% of the salary as the University allowance. An attractive package of associated benefit which includes superior furnished accommodation + free air tickets for him, his wife and 3 of his children (below 18 years).

REQUIREMENTS FOR APPOINTMENT:

*** PROFESSOR**
At least 10 years teaching experience post PhD with at least 2 years of which he spent in equal grade. Normally a minimum of 18 substantial publications in international referred journals or books in area of specialisation.

*** ASSOCIATE PROFESSOR:**
At least 6 years of teaching experience post PhD, 2 of which must be as associate Professor or equivalent grade. Normally a minimum of 6 substantial publications or research.

*** ASSISTANT PROFESSOR**
At least 4 years of teaching experience as assistant Professor or equivalent grade post PhD. Minimum of 3 substantial publications or research in the same area of specialisation being published in international referred journals or books.

*** DEPARTMENTAL SUPERINTENDENT:**
PhD + 10 years experience in the same specialisation or Master degree + 14 years experience or BSc + 18 years experience.

*** CHIEF TECHNICIAN:**
MSc + 10 year-experience in the same specialisation, or BSc + 12 year-experience at least.

Those who wish to apply for the above mentioned posts should write to:

Personnel Affairs Officer,
Sultan Qaboos University,
P.O. Box 32500, Al-Khod,
Muscat, Sultanate of Oman.

The following documents must be attached and enclosed:

1. Photocopies of Academic qualifications and experience certificates. 2. Curriculum Vitae (C.V.).
3. Passport Photocopy. 4. Passport size photographs 4 nos.

(W7616)A

UNIVERSITY of GUELPH

COLLEGE OF BIOLOGICAL SCIENCE

DEPARTMENT OF NUTRITIONAL SCIENCES

The Department of Nutritional Sciences invites applications for a tenure track position as an **Assistant Professor.** The position involves teaching undergraduate and graduate students and the development of an independent research program in nutritional sciences. A Ph.D. or equivalent degree in nutritional sciences or a related discipline such as biochemistry, cell biology, molecular biology, or physiology is required. Preference will be given to applicants with experience in teaching and post-doctoral research.

The Department of Nutritional Sciences concentrates on experimental nutrition at all levels of biological organization and is a member of the College of Biological Science which encompasses academic interests ranging from molecular biology to ecology. Examples of current research interests include the role of nutrients in biological membranes, in detoxification processes, in the control of inter-organ nutrient partitioning and in regulation of immune function.

The successful applicant will be expected to develop a laboratory based program to define processes by which health and development are influenced by diet and food. Examples of suitable subjects for such a program could be nutrition and neural function, aging, cancer, transport and cell communication.

Applications should include a curriculum vitae, a list of publications and names of three referees and be sent to: Dr. Henry S. Bayley, Department of Nutritional Sciences, University of Guelph, Guelph, Ontario, Canada N1G 2W1.

Closing date for applications is October 31, 1990.

The University of Guelph is committed to an Employment Equity Program which includes special measures to achieve diversity among its faculty and staff. In that spirit, we particularly welcome applications from qualified members of under-represented groups including aboriginal Canadians, persons with disabilities, members of visible minorities and women.

This position is subject to final budgetary approval. In accordance with Canadian Immigration requirements, priority will be given to Canadian citizens and permanent residents of Canada. (NW5594)A

HERIOT-WATT UNIVERSITY, EDINBURGH

POSTDOCTORAL RESEARCH IN ANALYTICAL/PHYSICAL CHEMISTRY

Applications are invited from physicists, physical or analytical chemists for a postdoctoral research associateship funded by the Ministry of Agriculture, Fisheries and Food. The successful applicant will work with Professor J Pfab on new wavelength selective HPLC detection techniques utilising laser excitation and supersonic jet-cooling. Previous experience with instrument development or with vacuum or laser spectroscopic techniques will be advantageous. The appointment wil be made for one year initially, but is extendable to two years.

Salary in the range of £11,399-£14,744 per annum depending on age and experience.

Please write (not 'phone) for an application form to the Staff office, Heriot-Watt University, Riccarton, Edinburgh EH14 4AS quoting ref no 94/90/N.

Closing date for completed applications: 5th September, 1990. (4318)A

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nature

THE INTERNATIONAL JOURNAL OF SCIENCE

seeks a

DEPUTY EDITOR

based in Washington, DC

Applications are invited for this post from persons with a background in the natural sciences who have:
*substantial experience of science journalism,
*respect within the research community
and who can write well in English.

The Deputy Editor will report directly to the Editor. The essential function of the post is to help to enhance the reputation and influence of *Nature* worldwide, but especially in the United States. Editorial input to some section of *Nature* will be an integral part of the job, as will be leadership and management of the Washington office.'

Salary from $60,000.

Applications, marked CONFIDENTIAL, should include a full *curriculum vitae* (resume) together with supporting material and should be sent to the Editor, John Maddox (at either the London or Washington offices) no later than 7th September, 1990.

(4335)A

MRC HUMAN BIOCHEMICAL GENETICS UNIT

Galton Laboratory, University College London

POST DOCTORAL RESEARCH POSITIONS

Three post doctoral research positions are now available in the MRC Human Biochemical Genetics Unit, Galton Laboratory, University College London. One is a joint MRC and Home Office funded 3 year project to study the molecular genetics of the human phosphoglucomutase gene family and the characterisation of alleles at the highly polymorphic PGM 1 locus by cDNA and immunological analysis. We wish to appoint a post doctoral scientist whose principal role will be to coordinate the production and characterisation of locus and allele specific monoclonal antibodies. Candidates should have a background in hybridoma technology and preferably but not necessarily have experience of epitope analysis using solid phase synthetic peptide technology.

The other two positions will also be 3 year short-term scientific staff appointments concerned with the core research programme of the Unit and related to the molecular and biochemical genetic characterisation of human enzymes and other proteins. The precise details of the projects will depend on the background experience and interests of the candidates appointed, but the most likely areas of research will be concerned with the carbonic anhydrase gene family and/or the intestinal disaccharidases, lactase and sucrase-isomaltase and/or the structure and function of hypervariable human mucin genes. Applicants for these posts must have a first degree in biology/biochemistry, genetics or equivalent and a PhD relating to molecular genetics.

Salaries will be in the range £14,148-£17,139 (incl LW, pay award pending). Applications including CV and details of 3 academic referees to Dr D A Hopkinson, Director, MRC Human Biochemical Genetics Unit, Galton Laboratory, UCL, Wolfson House, 4 Stephenson Way, London NW1 2HE. (4336)A

UNIVERSITY COLLEGE AND MIDDLESEX SCHOOL OF MEDICINE

Department of Immunology

RESEARCH ASSISTANT

Applications are invited for the post of Research Assistant (grade 1B) tenable for three years in the Department of Immunology. This Arthritis and Rheumatism Council funded project involves investigation of the genetic control of immunoglobulin glycosylation. Applicants should possess a degree in the biological sciences. Experience of molecular genetic techniques desirable. Salary scale £11,399-£13,495 plus £1,767 London Allowance. Applications including a c.v. and the names of two referees, should be sent as soon as possible to **Dr P.J. Delves (071-636 8333, ext. 3142 or 3139), Department of Immunology, UCMSM, Arthur Stanley House, 40-50 Tottenham Street, London W1P 9PG,** from whom further information can be obtained.

Equal opportunities employer. (4299)A

University of Waikato

Te Whare Wānanga o Waikato
Hamilton, New Zealand

SAPERE EST IUCUNDUM

SENIOR LECTURER/ LECTURER IN MEDICAL PHYSICS

The University invites applications for a lectureship or senior lectureship in the Department of Physics.

The appointee is expected to lecture and examine at all levels, including initial responsibility for the second year experimental physics course. The appointee will be expected to have research interests in medical physics. The interests of the Department in this field include imaging, ultrasonics, radiotherapy dosimetry and exercise quantification. A close relationship exists between the Physics Department and the physics and medical staff at Waikato Hospital. Projects are being carried out in conjunction with Waikato Hospital and with the Computer Science Department at the University of Waikato.

Other research interests of the Physics Department include plasma physics, electronics, quantum optics, physics education, astronomy and remote sensing.

Enquiries of an academic nature may be made to the Head of Physics Department, Professor B S Liley (Tel (64) 71 562889 Fax (64) 71 560115). Applications quoting reference number A90/69 close on 5th October 1990.

The current salary range per annum for Lecturers is NZ$36,000-NZ$47,200; and for Senior Lecturers is NZ$50,000-NZ$64,500.

Information on the conditions of appointment and method of application can be obtained from the Academic Staff Registrar, University of Waikato, Private Bag 3105, Hamilton, New Zealand. (Tel (064) 71 562889, Fax (064) 71 560135).

Places for appointees' children may be available in the creche run by the Campus Creche Society (Inc). The University welcomes applications from suitable people regardless of race, creed, marital status or disability. (W7612)A

KING'S COLLEGE LONDON
(University of London)

LECTURESHIPS IN IMMUNOLOGY

Applications are invited for two lectureships tenable from 1st October, 1990 in cellular/molecular immunology. One is under the New Academic Appointments Scheme, starting salary range £12,225-£17,139 inclusive (under review); the other is a temporary three-year post, salary £12,225-£22,236 inclusive (under review).

Further details, and application forms for return by 28th August, 1990, are available from Mr D.M. Drummie, Division of Biomolecular Sciences, King's College London, School of Life, Basic Medical and Health Sciences, Campden Hill Road, London W8 7AH.

Informal enquiries may be made to Professor R.M. Simmons on 071-836 8851 or Professor F.E.G. Cox on 071-836 5454. (4302)A

ENDOWED PROFESSORSHIP

The Department of Molecular Biology at Princeton University invites nominations and applications for an Endowed Professorship. Candidates should head an active research program and have an established reputation for excellence in scholarly research. Preference will be given to candiates with a strong background and research interest in biochemistry. For further information, contact:

Professor Thomas Shenk
Lewis Thomas Laboratory
Department of Molecular Biology
Princeton University
Princeton, New Jersey 08544

Princeton University is an Equal Opportunity/Affirmative Action Employer. (NW5565)A

THE UNIVERSITY OF BIRMINGHAM

CHAIR OF INORGANIC CHEMISTRY

Applications are invited for the established Chair of Inorganic Chemistry. This appointment, and one associated NAAS Lectureship, will sustain the research profile of the Inorganic Section of the School. The Chair will be vacant from 31 August 1990 and the aim is to have the new Professor in post as soon as possible after that date.

The current research strengths of the Inorganic Section are in co-ordination and metal-organic chemistry and in solid state chemistry.

Salary will be within the professorial range (minimum £27,013) plus superannuation.

Further particulars may be obtained from:

P.J.F. Scott, Director of Staffing Services,
The University of Birmingham, Edgbaston,
Birmingham B15 2TT. Telephone: 021-414 3842.

Closing date for applications 7th September 1990.

The University is an equal opportunities employer. (4304)A

IMMUNO

AKTIENGESELLSCHAFT FÜR
CHEMISCH-MEDIZINISCHE PRODUKTE

SENIOR BIOSTATISTICIAN

We are a producer and provider of ethical pharmaceuticals, biologics and diagnostics with international marketing and sales outlets. Our Clinical Research Group in Vienna, Austria has an opening for a Senior Biostatistician who will provide statistical consultation and collaboration on research projects, participate in the design, quality control, analysis and reporting of pre- and postmarketing clinical trials.

The successful candidate will be a native English speaker, has a PhD in Biostatistics/Statistics and several years experience in a similar position. Programming skills in SAS, BMDP and/or SPSS will be an added plus.

We offer a competitive salary and an excellent benefits package.

If you would like to be considered for this excellent opportunity please send your resume and salary history in strictest confidence to:

IMMUNO AG
Dr E. Pullez
Industriestraße 67
A-1220 Wien

(W7605)A

IMMUNO

Helix Biotechnology Ltd

A chance to get in on the ground floor of this newly established Biotechnology company.

Graduate Research Assistant in Molecular Genetics

Following our success in winning a DTI SMART Award, we wish to employ a graduate with skills in molecular genetics and protein purification. The project will involve taking novel enzymes from screening through to small-scale production. Available for one year. Starting salary at £11,583.

Graduate Research Assistant/ Technician

We are looking for a well organised and motivated graduate to carry out enzyme production work in our laboratories. Some practical experience of protein and nucleic acid purification is essential. Starting salary in £9,200 — £11,500 range with profit-related bonus.

Applicants should write (with c.v.) to **Dr Don Cowan, Helix Biotechnology Ltd, University College London, Gower St., London WC1E 6BT.** Enquiries can be made on (071) 387 7050 ext. 2246. (4340)A

UNIVERSITY OF ABERDEEN
LECTURESHIP IN BIOTECHNOLOGY

Applications are invited for a Lectureship under the UFC Biotechnology Initiative to be held as a joint appointment between the Departments of Zoology and Molecular and Cell Biology at the University of Aberdeen. Applicants should have interests in the application of molecular biology to the investigation of the physiology and immunology of both wild and farmed fish species.

Salary on the Lecturer scale: £12,086-£22,311 per annum.

Initial enquiries may be made to Professor Paul Racey (0224 272858) or Professor Ian Booth (0224 273152). Applicants will be invited to present a short seminar on their research on the day prior to interview.

Further particulars and application forms are available from the Personnel Office, University of Aberdeen, Regent Walk, Aberdeen AB9 1FX (tel: 0224 273500) to whom application (2 copies) should be returned by 24 August, 1990, quoting reference number JA/126. (4320)A

UNIVERSITY COLLEGE AND MIDDLESEX SCHOOL OF MEDICINE
Department of Molecular Endocrinology
POSTDOCTORAL RESEARCH ASSISTANT

Applications are invited for a postdoctoral research position from candidates with a minimum of two years' postdoctoral experience. The applicants must have experience in Northern blotting and/or *in situ* hybridisation techniques. An interest in neuroedocrinology although advantageous is not essential. The successful candidate should be able to work independently as well as part of a team investigating the placental control of foetomaternal thyroid hormone status and brain development using molecular biological techniques and protein fractionation biochemistry. The post is tenable for two years. Salary within the Research Assistant 1A scale: £13,166-£15,262 pa (incl L.W.). Applications to be submitted to: **Professor RP Ekins, Department of Molecular Endocrinology, UCMSM, Mortimer Street, London W1N 8AA, together with a CV and the names and addresses of two referees.** Closing date: 16th August, 1990.

Equal opportunities employer. (4300)A

POSTDOCTORAL POSITIONS

available only to citizens or permanent residents of the United States to work on structure, function and regulation of K+ , Na+ and Ca2+ channels, small and large G proteins, patch clamp and reconstitution.

Send CV to: **Dr Arthur M Brown, Chairman, Department of Molecular Physiology and Biophysics, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030. EOE.** (NW5589)A

GBF Gesellschaft für Biotechnologische Forschung mbH, Braunschweig, FR Germany — a government-supported Research Institute of Biotechnology — seeks a

MOLECULAR GENETICIST (POSTDOC)

for the Environmental Biotechnology Group of the Division of Microbiology.

The appointed person will genetically and biochemically analyse bacterial pathways for the degradation of aromatic environmental pollutants and the mechanisms of their regulation. The selective modification of regulator and enzyme functional parameters will constitute a central experimental strategy in this study and will be carried out in close cooperation with the protein design group of the GBF.

The position, which is available immediately, is for an initial period of two years.

Candidates should have a degree in genetics, microbiology or biochemistry and several years of experience (documented by appropriate publications in international journals) in gene regulation and/or the molecular genetic analysis of catabolic pathways. Experience in protein structure and function would be an advantage.

Send complete applications to GBF, Personnel Dept, Mascheroder Weg 1, D-3300 Braunschweig, FRG or contact Prof Kenneth Timmis (Tel: FRG 531/6181-400) for further information. (W7603)A

URBAN/PUBLIC HEALTH ENTOMOLOGIST

The Department of Entomology, University of Maryland, invites applications for a tenure track appointment as an

ASSISTANT PROFESSOR

Applicant must develop and implement innovative management programs for pests of structures and public health, conduct research in support of these programs, and teach an annual course in Urban Pest Management. Training in the principles and application of IPM is essential and experience with pests of structures and public health is desirable.

Send curriculum vitae, transcripts, statement of interests, and names and addresses of 5 references to: Dr Michael J Raupp, Department of Entomology, University of Maryland, College Park, MD 20742 by September 30, 1990. Telephone: 301-454-7025.

Equal Opportunity Employer/Affirmative Action. (NW5584)A

UNITED MEDICAL AND DENTAL SCHOOLS OF GUY'S AND ST THOMAS'S HOSPITALS
RESEARCH ASSISTANT

in analytical biochemistry required in the Department of Allergy, Guy's Campus. The department has a special interest in the role of lipoxygenase metabolites in health and disease. The applicant should have Ph.D. and previous experience in lipid biochemistry would be helpful. He/she would be part of a multi-disciplinary team studying the role of inflammation in the mechanisms of asthma and other allergic disorders. The successful applicant will be working closely with a synthetic chemist. GC-MS facilities are available.

Appointment for 2 years in first instance. Salary scale: £10,458-£16,665 pa plus £1,767 LWA. Further information from Professor T H Lee (071-955 4571) after 20 August.

To apply, please send 2 copies of CV, and names of 2 referees to The Personnel Officer, UMDS, St Thomas's Campus, Lambeth Palace Road, London SE1 7EH, quoting ref no G/MED/590. Closing date: 7th September, 1990. (4316)A

RESEARCH ASSOCIATE, RESEARCH ASSISTANTS

Human Genome Sequence: A new Biotechnology company located in Montreal, Quebec, Canada requires one Research Associate and two Research Assistants for November, 1990. Candidates must have experience in DNA cloning, phage and plasmid preparation, vector construction and DNA sequencing, and must be able to work interactively with a computer. In addition the successful applicant for the Research Associate position should have some supervisory experience and must be familiar with Pulsed-Field electrophoresis and PCR. Salaries are competitive and are according to qualifications and experience.

Please send applications, including a curriculum vitae and the names and addresses of two referees to **Dr R Preddie c/o Dr J E Bergmann, UKE, ZMK, Martinistr 52, 2000 Hamburg 20; West Germany.** (W7613)A

Within the field of healthcare research and product development, no name carries more prestige than Amersham.

PRINCIPAL CLINICAL ENDOCRINOLOGIST

c£23,000

Cardiff

An acknowledged leader in many specialist areas, we are currently making major breakthroughs in the provision of diagnostic tests for thyroid conditions, and it's within the clinical reagents division that we're looking to appoint a Principal Clinical Endocrinologist.

Based at Cardiff, in purpose built facilities, you will be primarily responsible for the support, development and enhancement of a range of clinical assays. Much of the work will involve the development of close working relationships with leading clinicians in the UK and abroad to enhance the company's position in thyroid endocrinology.

It's a demanding role, and our requirements are understandably stringent. A post-doctoral senior biochemist, or medically qualified research scientist, you will already have extensive experience in clinical chemistry.

The challenge is matched only by the rewards – which include a highly attractive salary, generous relocation expenses and a wide range of other large company benefits.

Applications should be made to Martyn Bishop, Personnel Manager, Amersham International plc., Clinical Reagents, Forest Farm, Whitchurch, Cardiff CF4 7YT, quoting reference CLIN1. Closing date for applications: 31 August 1990. Telephone 0222 615858 ext 3230.

(4343)A

Bringing science to life

Amersham

En raison de l'élection du Prof. H. Meier, Directeur de l'Institut de Biologie végétale à la Faculté des Sciences de l'Université de Fribourg (Suisse), comme Recteur de l'Université de Fribourg dès le printemps 1991, la Faculté des Sciences met au concours un poste de

Professeur de Biologie végétale, Directeur de l'Institut de Biologie végétale et de Phytochimie

La/le titulaire de la chaire est responsable de l'administration de l'Institut, de la coordination de l'enseignement et de la recherche, de même que de la planification à terme des branches qui y sont représentées. Outre la participation à une partie de l'enseignement, la/le candidat/e doit pouvoir justifier d'une recherche originale, active et compétitive dans les domaines de la biologie végétale moléculaire ou de la physiologie des plantes. L'enseignement doit être fait en français ou en allemand, de bonnes connaissances de la langue complémentaire sont exigées.

Les candidat(e)s sont prié(e)s d'adresser leur dossier (curriculum vitae, liste des publications, copies de trois travaux parmi les plus remarqués, un résumé de une à deux pages de leur recherche en course et à venir, trois références) au Décanat de la Faculté des Sciences de l'Université de Fribourg, Pérolles, CH-1700 Fribourg, jusqu'au 15 novembre 1990.

Le Décanat se tient à disposition pour de plus amples informations. (W7604)A

INSTITUTE OF DENTAL SURGERY

Department of Maxillofacial Surgery and Oral Medicine

RESEARCH ASSISTANT

Required as soon as possible to study the role of cytokines in the pathogenesis of experimental arthritis. The work will involve cytokine assays, immunocytochemistry and *in situ* hybridisation and measurement of connective tissue metabolism. The position is funded by the Oliver Bird Fund for Research into Rheumatism (Nuffield Foundation).

Candidates should possess a good honours degree and some laboratory experience. Initial salary up to £12,855 (under review). Applications, including a full CV and the names, addresses and telephone numbers of two academic referees should be sent to The Personnel Department, Eastman Dental Hospital, 256 Gray's Inn Road, London WC1X 8LD.

(4293)A

UNIVERSITY OF DUNDEE

Department of Biochemistry

POSTDOCTORAL POSITIONS IN NUCLEIC ACID STRUCTURE AND DNA-PROTEIN INTERACTIONS

The laboratory of David Lilley is making significant progress in understanding the structures of a number of branched DNA structures and their interactions with enzymes and other binding proteins, RNA structure and the cellular role of DNA supercoiling. A number of postdoctoral positions are currently available in the following areas:

1. The genetics of bacteria and yeast; expression and mutagenesis of proteins in bacteria.
2. DNA enzymology and protein purification.
3. NMR spectroscopy and DNA and RNA structures.

While these are preferred areas of expertise, we would be pleased to hear from anyone with general interests in nucleic acid structure and function. We are well equipped for these studies, including facilities for DNA and RNA synthesis and purification, 500 MHz NMR, Evans and Sutherland computer graphics. Several of our areas of interest involve callaborations with other European laboratories, with whom there is frequent contact. In addition, Dundee Biochemistry Department is a centre of excellence, with a UGC5 rating.

Informal enquiries may be made to Professor Lilley (0382) 23181, ext. 4243.

Applications in writing, with full CV (2 copies) including the names and addresses of two referees to, The Personnel Office, The University, Dundee, DD1 4HN. Please quote reference EST/407/90/N.

Closing date: 23rd August, 1990. (4341)A

UNIVERSITY OF EDINBURGH

CHAIR OF TROPICAL ANIMAL HEALTH

Applications are invited from suitably qualified candidates for the above post in the Faculty of Veterinary Medicine. It is hoped that the successful candidate will be able to take up the appointment from 1st April, 1991. The salary will be in the professorial range.

Further particulars may be obtained from the Secretary to the University, University of Edinburgh, 63 South Bridge, Edinburgh EH1 1LS, with whom applications (13 copies), including curriculum vitae and the names of three referees, should be lodged not later than 5th October, 1990. Overseas candidates need submit only one copy of the application.

Please quote reference no: 89/90. (4296)A

CHIEF RESEARCH TECHNICIAN

Current opening for a Chief Research Technician in the Biomedical Physics Department of the King Faisal Specialist Hospital and Research Centre in Riyadh, Saudi Arabia. Must be capable of supporting all aspects of mammalian cell cultures including in vivo/in vitro systems. Experience in radiation biology would be desirable. Minimum of 5-7 years mammalian research experience with BS degree in appropriate science (some reduction of experience requirement possible with MS degree). Potential tax-free salary and excellent benefits.

Send resume and salary requirements to: **Hospital Corporation International, 2515 Park Plaza, Nashville, TN 37203,** or call toll-free **1-800-251-2561** in the US, **1-800-342-2110** in Tennessee, or COLLECT at **615-320-2440** from Canada. HCI is an Equal Opportunity Employer.

(NW5588)A

THE UNIVERSITY OF PENNSYLVANIA, Children's Hospital of Philadelphia

is recruiting for a

PROFESSOR

In the Division of Human Genetics and Molecular Biology

The position is for a molecular-cytogeneticist and molecular-biologist who is expected to direct an independent Cytogenetics Research Program with a focus in genome mapping and cancer/constitutional cytogenetics research. This person will also be repsonsible for a Clinical Cytogenetics Laboratory and a Cytogenetics Core facility for The Children's Hospital of Philadelphia.

The position is tenured and will be filled by an established scientist with expertise in molecular genetics, molecular-cytogenetic analysis of chromosomal abnormalities and somatic cell genetics.

Certification as a Clinical Cytogeneticist and a PhD Geneticist or Clinical Geneticist by the American Board of Medical Genetics are required.

Send curriculum vitae to: **Dr Michael Zasloff, Division of Human Genetics and Molecular Biology, The Children's Hospital of Philadelphia, 34th Street and Civic Center Blvd, Philadelphia, PA 19104.**

The University of Pennsylvania is an equal opportunity, affirmative action employer. (NW5585)A

UNIVERSITY OF LONDON

Department of Biochemistry and Biological Sciences
Wye College

LECTURESHIP IN PLANT MOLECULAR BIOLOGY

Applications are invited for a Lectureship in Plant Molecular Biology which is available from 1st October, 1990. The post, which is supported by the UFC Biotechnology Initiative over a period of five years subject to review after three years, is to develop teaching and research into the application of transgenic plant technology to species of agricultural significance.

The appointment will be made on the Lecturer Grade A scale, currently £12,086 to £16,755.

Applications with a full curriculum vitae and names of at least two referees should be sent to: Dr T.A. Hill, Assistant Secretary, Wye College, University of London, Ashford, Kent TN25 5AH, U.K. from whom further particulars can be obtained. Tel: 0233 812401. Fax: 0233 813320.

The University follows an equal opportunity policy. (4294)A

UNIVERSITY COLLEGE AND MIDDLESEX SCHOOL OF MEDICINE

Department of Medicine

POSTDOCTORAL SCIENTIST

An enthusiastic scientist is needed to join a group working on the molecular and cell biology of a recently-cloned membrane protein, which is absent in a rare inherited haemolytic anaemia. The cells show a marked "leak" to sodium and potassium and the protein may prove to have an important role in the regulation of ionic movements. The aims of the work will be to elucidate the structure and function of the protein, the genetic abnormalities in the patients and the mechanism of the functional abnormality in the cells. Prior experience of DNA or protein techniques will be useful but not essential.

The post is funded by the Wellcome Trust and is for three years in the first instance. The salary is on the 1A scale (£11,399-£18,165) plus London Weighting £1,767).

Enquiries should be made to Dr Gordon Stewart on 071-380 9725 or in writing to the Department of Medicine, Rayne Institute, University Street, London WC1E 6JJ. Equal opportunities employer.

(4298)A

FACULTY POSITION AVAILABLE

Gastroenterology/hepatology

The Department of Medicine, Division of Gastroenterology at the University of California, Los Angeles, invites applications for a tenure-track or tenure faculty position that is currently available at the Assistant or Associate Professor level. Applicants must have either an MD or MD/PhD degrees. Research and clinical experience in hepatology is preferred. Newly built laboratory space, "start-up funds," and an excellent research environment will be provided.

Please send curriculum vitae, a brief letter describing your research program, and the names of three references to: **Dr Robert I Lehrer, Department of Medicine, UCLA School of Medicine, Los Angeles, CA 90024-1678.**

UCLA is an Affirmative Action/Equal Opportunity Employer.

(NW5590)A

THE UNIVERSITY OF BIRMINGHAM

Department of Physiology

POSTDOCTORAL RESEARCH FELLOW

This three year position is funded by the Motoneurone Disease Association and is available for the study of neuropeptide expression in regenerating nerves. Experience in biochemical separation techniques, tissue culture, or current techniques in molecular biology an advantage.

Informal enquiries to Dr M E Smith on 021-414 6903.

Salary on the range £11,399-£18,165.

Application forms (returnable by 23rd August 1990) and further particulars available from the Director of Staffing Services, The University of Birmingham, Edgbaston, Birmingham B15 2TT. Tel: 021-414 6483 (24 hours). Quote ref: M12064.

THE UNIVERSITY IS AN EQUAL OPPORTUNITIES EMPLOYER

(4334)A

UNIVERSITY OF LONDON
Department of Biochemistry and Biological Sciences
WYE COLLEGE
POSTDOCTORAL RESEARCH ASSOCIATE IN MEMBRANE PHYSIOLOGY

Applications are invited for a Postdoctoral Research Associate, funded by the AFRC, to explore mechanisms of NO_3 transport across plant and fungal cell membranes.

The post is available from 1st October, 1990 for three years, starting at point 6 on the RA 1A scale (£13,495 to £14,744).

The successful applicant will undertake electrophysiological studies of NO_3 transport using intracellular microelectrodes and will also help to maintain cell cultures. Previous experience in at least one of the areas, either (1) in intracellular recording with single cells, eg voltage clamping and use of ion-sensitive microelectrodes or (2) in plant cell culturing, is desirable.

Applications with a full curriculum vitae and names of at least two referees should be sent to: Dr T A Hill, Assistant Secretary, Wye College, University of London, Ashford, Kent TN25 5AH, UK from whom further particulars can be obtained. Tel: 0233 812401, Fax: 0233 813320.

The University follows an equal opportunity policy. (4342)A

UNIVERSITY OF WALES COLLEGE OF MEDICINE
Departments of Medical Genetics and Psychological Medicine
RESEARCH OFFICER (MOLECULAR GENETICS)

Research Officer required to work on a molecular genetic study of schizophrenia. The work will involve RFLP analysis and the use of PCR to detect microsatellite polymorphisms. The work would suit a recent graduate in biochemistry, genetics, biology or a related subject. The post is available from 1st September 1990 and is funded initially for two years by the WSDHSR. The opportunity to register for a PhD may be available.

Starting salary £11,399 on the scale for University Research and Analogous Staff Grade 1B (£11,399-£13,495 per annum).

Further details (quoting Ref No M8D/1/27) available from the Personnel Office, University of Wales College of Medicine, Heath Park, Cardiff CF4 4XN (Tel: 0222 755944 ext 2296) to whom applications in the form of a curriculum vitae with the names and addresses of two referees should be submitted by 17th August, 1990. (4324)A

IMPERIAL COLLEGE
(University of London)
RESEARCH ASSISTANT AIR POLLUTION

A 2 year post-doctoral post, funded by NERC, is available in the Air Pollution Group at Imperial College, London, for modelling studies of the influence of ammonia on aerosol formation and long-range atmospheric transport of sulphur and nitrogen species, This is a pilot project within a new European collaboration to characterise aerosol burdens over Europe, to be coordinated under the EUROTRAC programme on atmospheric chemistry.

Applications with C.V., and further information from: Dr HM ApSimon, Air Pollution Group, Mech, Eng. Dept., Imperial College, London SW7 2AZ, United Kingdom. (4315)A

Please mention

nature

when replying to these advertisements

ASSISTANT PROFESSOR

The Biology Department, Utah State University, Logan, Utah, is seeking a tenure-track Assistant Professor with research specialty in Plant Pathology. Research involving current technologies on fungal pathogens relevant to agriculture in Utah is required. The position involves 50% research and 50% teaching responsibilities (three courses per year) that include Mycology and Introduction to Plant Pathology. Excellence in teaching and research is expected. Please send curriculum vitae, three letters of reference and a statement of research goals to Dr Anne Anderson, Department of Biology, Utah State University, Logan, UT 84322-5305. Review of the applications will begin October 1, 1990, and continue until the position is filled.

USU is an AA/EO Employer. (NW5591)A

THE BRITISH SOCIETY FOR HORTICULTURAL RESEARCH, LITTLEHAMPTON
Entomology & Insect Pathology
TEMPORARY HIGHER SCIENTIFIC OFFICER

A Higher Scientific Officer is required for up to three years to work on the integrated and biological control of insect pests in glasshouses; to specialise on the development of Integrated Pest Management systems for application to ornamental crops under glass and to supervise other staff working on externally-funded contracts.

Qualifications: First or Upper class Hons degree in a life science subject. Ph.D. degree in entomology or crop protection topic desirable; driving licence required. Salary in range £10,678-£14,909.

Further details and application form from the Personnel Officer (Miss M Borner), British Society for Horticultural Research, East Malling, West Malling, Kent ME19 6BJ. Ref 11/L/1. Closing date 24th August, 1990.

The BSHR is an Equal Opportunities Employer. (4313)A

FELLOWSHIPS

UEA
NORWICH
POSTDOCTORAL FELLOWSHIPS
Molecular Evolutionary Biology

These SERC-funded posts are available for three years in the School of Biological Sciences. Using PCR to investigate rDNA and scDNA sequence variation in hybrid zones in grasshoppers and the phylogenetic variation in Europe. The University adjoins the John Innes Research Institute and other biological Institutes providing a range of molecular and population biology expertise. Candidates should hold or actually be submitting a PhD.

They should apply as soon as possible, sending a curriculum vitae with names, addresses and telephone numbers of referees, to **Professor G M Hewitt, School of Biological Sciences, University of East Anglia, Norwich NR4 7TJ, UK.**

UEA is an equal opportunities employer. (4332)E

THE UNIVERSITY OF BIRMINGHAM
School of Biological Sciences
NERC POSTDOCTORAL RESEARCH FELLOWSHIP

Growth and Reproductive Strategies of Phagotrophic Microflagellates in Marine Foodwebs.

This project (tenable for 3 years) involves laboratory experiments using batch and continuous culture systems in which competitive interactions between predator flagellates for common prey provided under various conditions will be investigated. Field work will be carried out in conjunction with Plymouth Marine Laboratory and will include work on field samples and a sea-going cruise.

Experience in modelling predator/prey dynamics desirable and experience in Phytoplankton ecology or aquatic microbiology desirable but not essential.

Salary in a range £11,399-£18,165.

Application forms (returnable by 24th August 1990) and further particulars available from the Director of Staffing Services, The University of Birmingham, Edgbaston, Birmingham B15 2TT. Tel: 021-414 6483 (24 hours). Ref: S13084.

The University is an equal opportunities employer. (4333)E

NEW ZEALAND MINISTRY OF AGRICULTURE AND FISHERIES

FELLOWSHIP

Physiology and Molecular Biology of Fibre Growth

Ruakura Agricultural Centre, Hamilton,
New Zealand. Vacancy No. MTN 90/48

Applications are sought for a scientist to join a research team working on the physiology of animal fibre growth. The Physiologist/Molecular biologist appointee will initiate and carry out research projects related to the endocrinology and the cell biology of seasonal hair and wool growth. Research experience in one or more of the following fields is necessary: cell physiology, molecular biology, endocrinology or animal physiology. The minimum qualifications are an Honours degree with 3-5 years relevant experience or a Ph.D.

The Ruakura Agricultural Centre has access to unequalled animal resources and has excellent facilities for studies involving animal physiology, molecular biology and genetics. The work is allied to other Ruakura programmes involving the physiology and molecular biology of lactation, muscle growth and reproduction. Ruakura is situated in pleasant rural surroundings adjacent to the University of Waikato and the city of Hamilton (population 100,000). The programme provides an excellent opportunity to work in an expanding basic research programme with considerable applied potential.

The appointment will take the form of a MAF Fellowship for an initial 3 year period with the right to negotiate an extension at the end of the second year. Salary will be in accordance with experience and qualifications but would normally be in the range of $NZ33,000-50,000. Travel expenses will be covered under the Fellowship.

Further information can be obtained from Dr Allan Pearson, Ruakura Agricultural Centre, Private Bag, Hamilton, New Zealand. Telephone +64 71-562 839; Fax +64 71-385 658.

Applicants should send a curriculum vitae including academic record, publication list, employment record and the names of two referees (quoting vacancy number MTN 90/48) to: Management Support Officer, Animal Research Group, MAF Technology, Ruakura Agricultural Centre, Private Bag, HAMILTON, New Zealand.

Applications close on 17th September, 1990. (W7615)E

ROYAL COLLEGE OF PHYSICIANS OF EDINBURGH

JOHN, MARGARET, ALFRED AND STEWART SIM FELLOWSHIP

Applications are invited from graduates in medicine or in one of the basic sciences applicable to medicine for a second Sim Fellowship for research into biochemical aspects of depressive illness.

The Fellowship will be for a period of three years in the first instance, the first of which will be a probationary year. Salaries will normally be on the University lecturers' scales, clinical or non-clinical as appropriate. Provision for technical support and expenses can be provided. The Fellowship will normally be based in Edinburgh, but some of the work may be carried out in departments outside Edinburgh if this seems appropriate to the College.

Applications should include a curriculum vitae and a statement of about one thousand words outling the aims, background and plan of the proposed research. Applications should also include the names of three professional referees and be accompanied by a letter from the head of the host department showing willingness to provide the necessary space and facilities for the Fellow.

Further details and an application form are available from: The Treasurer, Royal College of Physicians, 9 Queen Street, Edinburgh EH2 1JQ, to whom all correspondence should be addressed. The closing date for applications for the award is 30th November 1990. (4331)E

Leicester University

Departments of Biochemistry and Genetics

RESEARCH FELLOWSHIP IN TRANSGENIC ANIMAL TECHNOLOGY

Applications are invited for a five-year Research Fellowship in transgenic animal technology. This is a joint appointment between the Departments of Biochemistry and Genetics, funded by earmarked industrial sponsorship. The two closely aligned Departments have strong research records and a common interest in utilising and developing transgenic techniques to study mammalian gene-expression and function.

Post-doctoral candidates with experience of molecular biology and transgenic animal techniques will be welcomed. The necessary equipment, facilities and basic running costs are available. The successful candidate will be encouraged to develop his/her own research projects, but there are opportunities for interaction with other groups interested in gene-manipulation in ES cells and the construction of transgenic models of human diseases.

The salary will be on the 1A scale, £11,399 to £18,165, and the appointment is available from 1st October, 1990.

Applications, including a curriculum vitae of two academic referees, should be sent to **Professor W J Brammar, Department of Biochemistry, University of Leicester, University Road, Leicester LE1 7RH, by 23rd August, 1990.** (4290)E

EMBO

EUROPEAN MOLECULAR BIOLOGY ORGANIZATION

SHORT TERM FELLOWSHIPS in molecular biology

The European Molecular Biology Organization awards, to scientists working in Europe and Israel in the field of molecular biology and allied disciplines, short term fellowships of one week up to three months duration. The fellowships are to support collaborative research between laboratories in different countries and provide a travel grant and subsistence allowance. Applications may be made at any time and are decided upon soon after the receipt of application.

Applications for exchanges between laboratories within any one country cannot be considered. Fellowships involving transatlantic travel are awarded only in extremely exceptional circumstances. Inquiries should be accompanied by a self-addressed adhesive label.

Application forms and further details may be obtained from Dr J. Tooze, Executive Secretary, European Molecular Biology Organization, 69 Heidelberg 1, Postfach 1022.40, F.R.G. (W6896)E

NEUROSCIENCE TRAINING PROGRAM

The University of Connecticut Health Center invites applications for postdoctoral fellowships in communicative sciences (hearing and chemical senses), molecular, cellular, and neural biology, an interdisciplinary, multidepartmental program.

Open to US citizens or permanent residents.

Write to: Dr D Kent Morest, Center for Neurological Sciences, University of Connecticut Health Center, Farmington, CT 06032. Telephone: 203-679-4678.

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Candidates with at least an upper second class honours degree in Biochemistry, Modecular Biology, Cell Biology, or a relevant area should send a C.V. and the names of 2 referees to **Dr. P Goldfarb, Department of Biochemistry, University of Surrey, Guildford, Surrey GU2 5XH. (Tel. 0483 571281 ext. 2534).** (4338)F

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The Department has particular emphasis at the organismal level in aquatic science (marine and freshwater), environmental physiology, behaviour, parasitology, wildlife biology, and resource management.

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Applications, including a curriculum vitae, and the names of three referees should be forwarded by September 30, 1990, to: Dr. Bruce H. Sells, Professor and Dean, College of Biological Science, University of Guelph, Guelph, Ontario, Canada N1G 2W1. (NW5595)N

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120 YEARS AGO

PROF. Wanklyn says the opponents of spontaneous generation are placed in a dilemma by some new facts and arguments recently advanced in favour of the theory by Dr. C. Bastian, whose observations were only concluded in *Nature* for July 14, 1870.

Prof. Wanklyn might have allowed the "opponents" a little more breathing time, and if his cause is really as strong, why so soon call attention to "other difficulties" under which they labour? These tactics compel me to remark that the spontaneous generation theory will gain nothing by being forced upon the attention. Like other doctrines it must stand or fall according as the facts on which it is based are confirmed or refuted... Prof. Wanklyn is rather damaging the cause he hopes to serve in another way. He states that "rather more than one pint of average atmospheric air does not contain so much organic nitrogenous matter as corresponds to a cube of dry albumen of the 1/250th part of an inch in diameter," and affirms that this quantity is altogether inadequate to account for the "immense multitudes of germs, the existence of which in atmospheric air is assumed by the vitalists." Now these "vitalists" hardly require "immense" multitudes, and instead of being "assumed," the presence of quite a sufficient number has been *proved*, and moreover, they can be seen by anyone who chooses to take the trouble to look for them.

But I thank Prof. Wanklyn for his fact and calculation. The evidence he adduces is very interesting, and conclusively in favour of the presence of living germs in the air; for those who accept the views he objects to, do not require a tithe or even a hundredth part of the albuminous matter he finds actually exists. The space occupied by a moist blood corpuscle 1/3000th of an inch in diameter, which only contained even less than a *tenth of its weight* of dry alluminous matter, would be large enough to hold so many living germs that if uniformly diffused through the pint of air, not a thimbleful could be taken without containing several. These germs, placed under favourable circumstances, would soon grow large enough to be detected without any difficulty.

'Spontaneous Generation', Lionel S. Beale.
From *Nature* July 28, 1870.

100 YEARS AGO

WE learn from the *Bulletin* of the Torrey Botanical Club that, through the cordial co-operation of the officers of the New York State Fish Commission, and the great personal interest of its President, Mr. Eugene Blackford, the Brook's Institute has been enabled to open a sea-side laboratory for teaching and research in zoology and botany, under the direction of Dr. Bashford Dean. The laboratory is located at Cold Spring Harbour, Long Island, 32 miles from New York, reached by the Long Island Railway. The session opened on July 7, and was to extend over eight weeks; the fee is 24 dollars. The location is described as a capital one, and an extensive corps of lecturers on special subjects has been secured, those on the botanical side being Dr. W. G. Farlow, Dr. N. L. Britton, and Prof. Byron D. Halsted.

IN his recent thesis on the influence of the sea-shore on leaves M. Pierre Lesage shows by conclusive evidence that a marine habitat leads to a thickening of the leaves. The palissade-cells of plants from such a habitat are more numerous and larger than in the leaves of the same plants inland. Apparently the sea-salt is the cause of this alternation, as plants cultivated in artificially salted soil yield thicker leaves. The observations of M. Lesage bear on some ninety species of plants which are in their natural state found near the sea (in Brittany) as well as inland.
From *Nature* July 31, 1890.

50 YEARS AGO

DEMONSTRATIONS of the stereophonic reproduction of music and speech were given at the Carnegie Hall in New York City on April 9 and 10. This was the culmination of a long series of researches by Bell Telephone Laboratories. The first step in this achievement was demonstrated in 1933, when a symphony concert produced in Philadelphia was transmitted over telephone wires to Washington and there reproduced stereophonically and with enhancement before the National Academy of Sciences. Subsequent researches by Dr. H. Fletcher and his colleagues at the Bell Laboratories have supplied the equipment and technique for recording such a production on film.

With the co-operation of Leopold Stokowski and the Philadelphia Orchestra, the Tabernacle Choir and organists in Salt Lake City, and of Paul Robeson and other artists, their music and drama was recorded both in Philadelphia and in Salt Lake City. At a later audition, the director or artist was able to vary the recorded volume and to change the tonal colour of the music to suit his taste. He could soften it to the faintest pianissimo or amplify it to a volume of ten times that of any orchestra without altering its tone quality, or he could augment or reduce the high or low pitches independently. While he was thus enhancing the music he himself had directed, his interpretation was re-recorded on film as a permanent record.

Selections for reproduction, chosen to demonstrate the full capabilities of this system, were given. A stirring climax for the programme was provided by the closing scene of the "Götterdämmerung". Dr. Stokowski took full advantage of the tenfold increase of sound over that of the largest orchestra, and effectively used the individual control to make the soloists' voice clearly heard above the orchestra. Dr. Stokowski has shown a full grasp of the possibilities of the new system.

"Stereophonic Records of Enhanced Music".
From *Nature* August 3, 1940.

10 YEARS AGO

A LIZARD of the iguanid genus *Anolis* enclosed in amber of Oligocene or Miocene age from the Dominican Republic represents the first complete vertebrate fossil in amber as well as the oldest and only complete fossil of its genus. The fossil *Anolis* is very closely related to a recent form and will shed some light on the evolution of its genus which otherwise has a poor fossil record.

In view of its uniqueness I propose to name the fossil anole *Anolis dominicanus* n. sp. The fossil *Anolis* has a total length of approximately 71 mm; the tail is complete and not regenerated. The total length of the specimen indicates either a juvenile or a small species.

"Green anole in Dominican amber", Olivier Rieppel.

SIR—I suggest that we generalize the convention implicit in the naming of the Earth's year. We suffer from a surfeit of planetary adjectives — should one speak of the mercuric or mercurial year, the saturnian, saturnine or saturnalian? Can one, in a reputable journal, mention the venereal or uranal year? How much simpler to name the planetary years *ymer, yven, year, ymar, yjup, ysat, yura, ynep* and *yplu*. How fortunate that y is both vowel and consonant.

"Adjectival concerns", Peter Rastall.
From *Nature* July 31, 1980.

IN NEXT WEEK'S ISSUE

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All contributions submitted for publication in *Nature* should conform with these rules.

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Artwork should be marked individually and clearly with the author's name, figure number and, when known, manuscript reference number. Original artwork should be unlettered. Ideally, no figure should be larger than 28 by 22 cm. Figures with several parts are permitted only if the parts are closely related, either experimentally or logically. Suggestions for cover illustrations, with captions, are welcome. Original artwork (and one copy of the manuscript) will be returned when a manuscript cannot be published.

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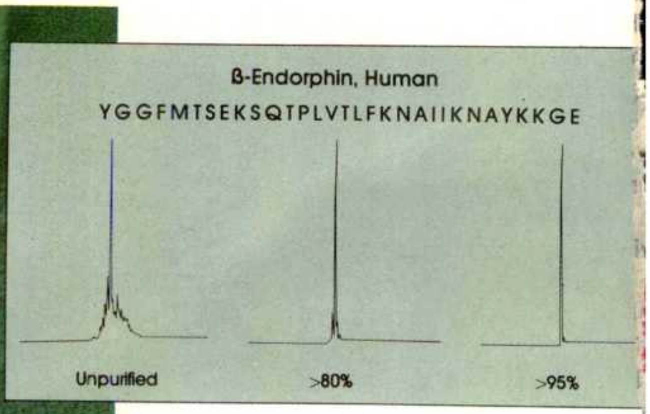

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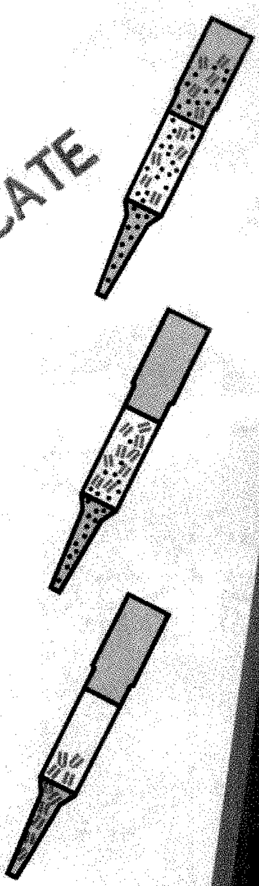

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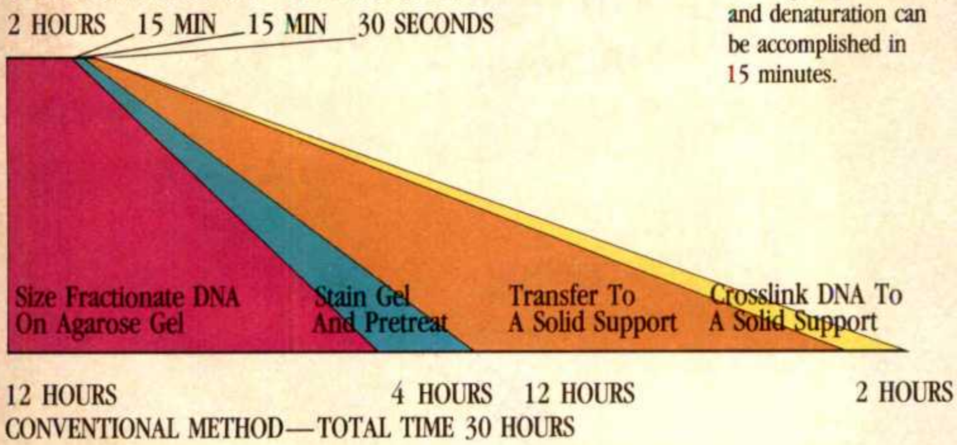

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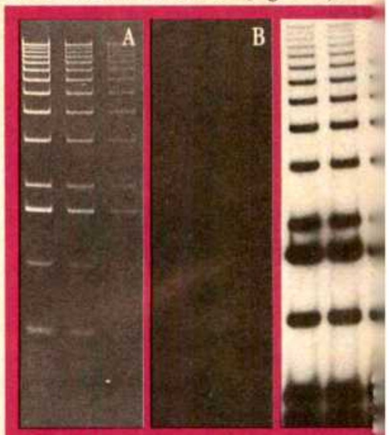

FIGURE 1:

Figure Legend: Fractionation of end labeled DNA markers on 3mm thick 0.8% agarose by the VAGE apparatus and transfer to Duralon—UV™ membranes using the PosiBlot pressure blotter.

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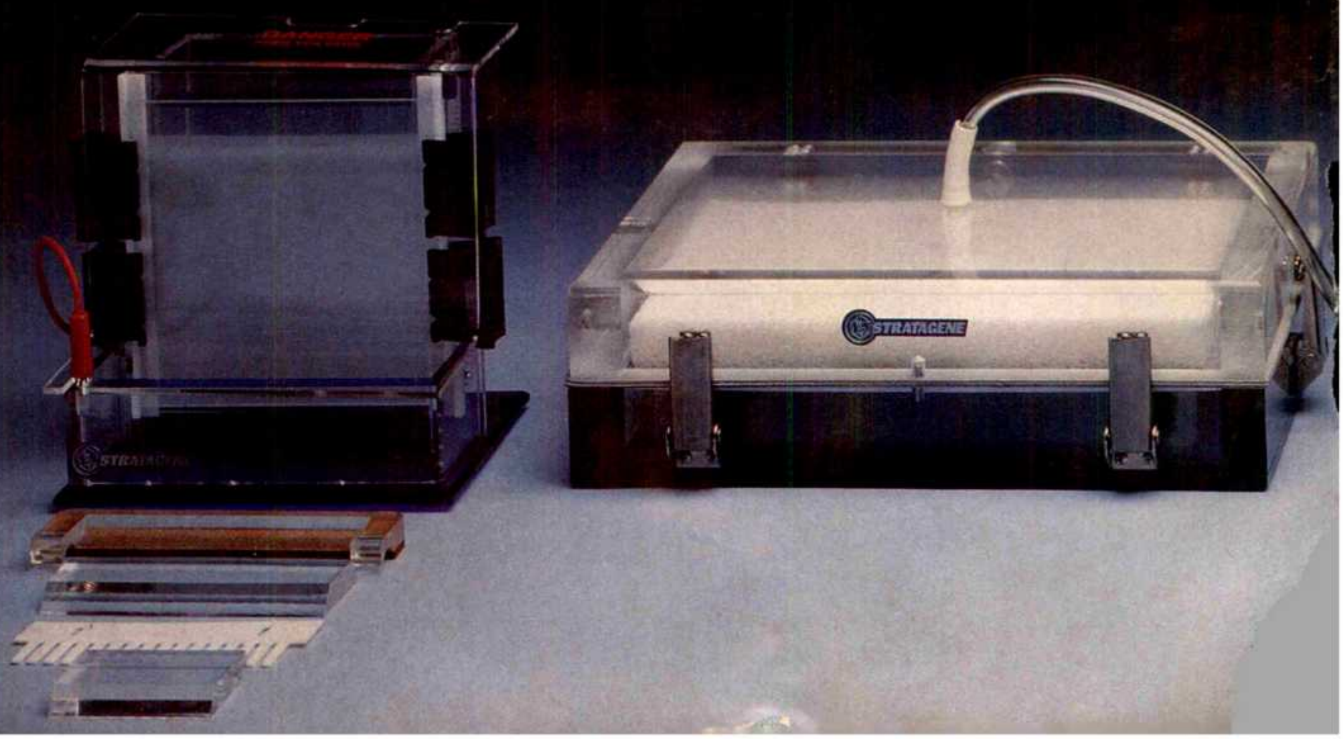

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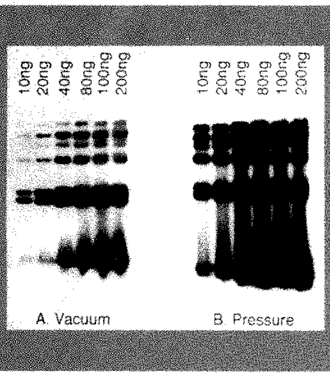

FIGURE 2:
Figure Lengend: ^{32}P end-labeled lambda Hind III markers were electrophoresed in 0.8% agarose. The DNA was then transferred to a nylon membrane with a vacuum blotter at 30mm Hg below atmospheric or with the PosiBlot pressure blotter at 100mm Hg above atmospheric. Both transfers were carried out for 15 minutes. As can be seen, pressure blotting transferred significantly more DNA in the same period of time, especially in the higher molecular weight range (largest band is 23 kilobases).

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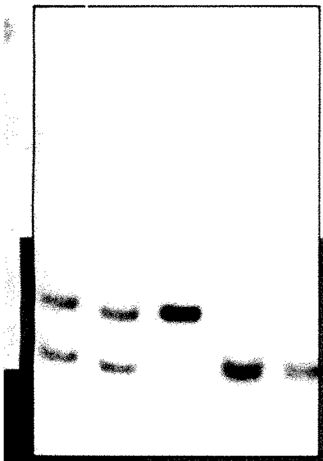

FIGURE 3:
Figure Legend: Autoradiogram showing the resolution of 2.8 and 1.3 Kb Msp I RFLP alleles revealed by a cystic fibrosis human DNA probe using the VAGE, PosiBlot and Stratalinker all in 2.5 hours.

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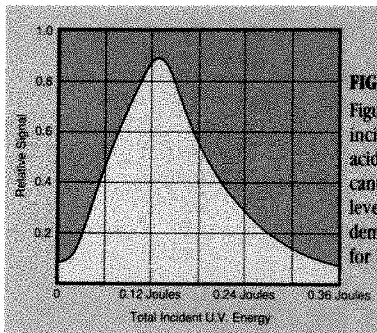

FIGURE 4:
Figure Legend: The effects of altering the incident energy for crosslinking nucleic acids to nylon membranes. The significant drop in signal intensity at energy levels below and above 0.12 Joules demonstrates the limited optimal range for UV treatment.

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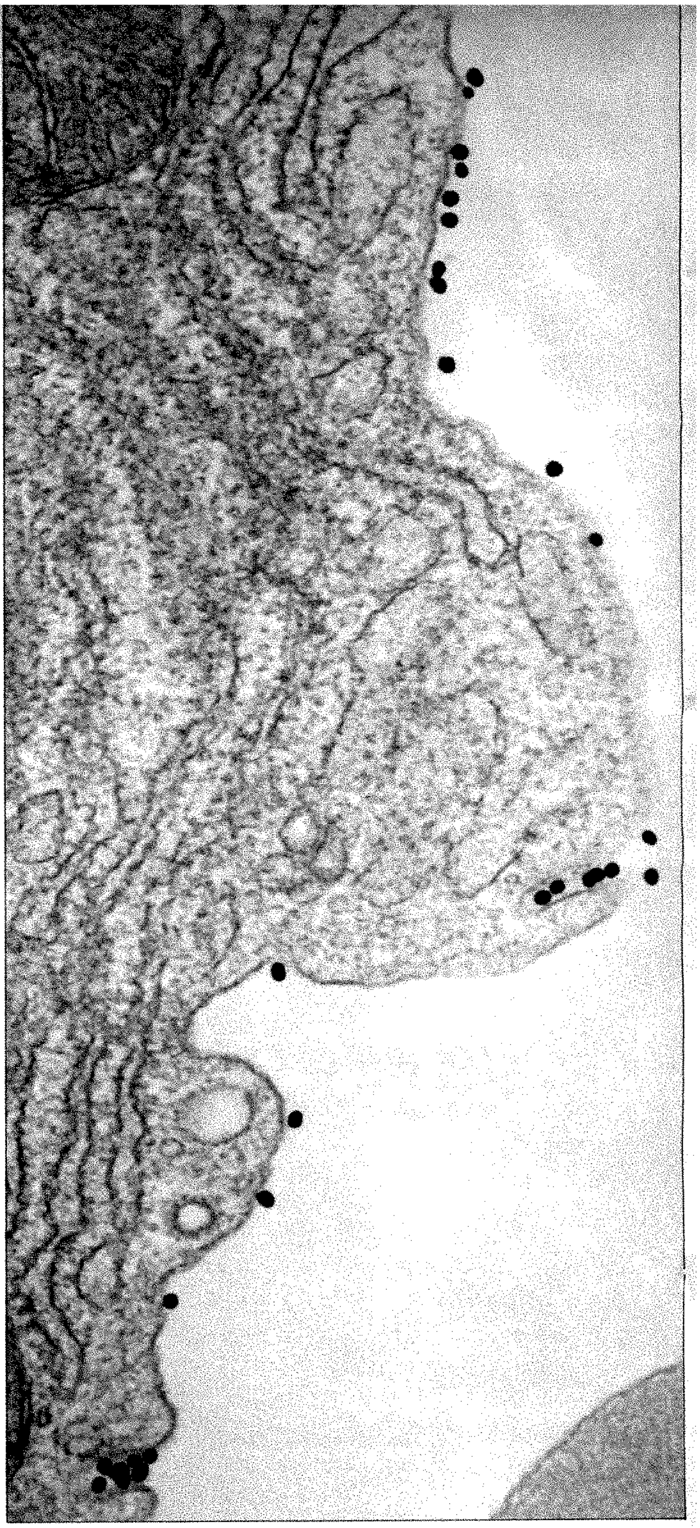

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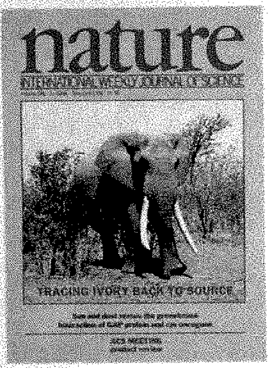
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◀ Analysis of carbon, nitrogen and strontium isotopes in ivory and bone could provide a means of identifying the area from which individual elephant tusks were derived and thus be a valuable tool in the fight to combat ivory poachers and to conserve the African elephant. Pages 744 and 747. Photo: A. Hall-Martin.

THIS WEEK . . . THIS WEEK . . . THIS WEEK . . .

Heat and dust

The extent to which global warming might be moderated by variation in solar radiation and atmospheric dust is considered in this week's Review Article. The conclusion is that solar variability will not counteract warming, and that despite uncertainties in the effects of dust, greenhouse-gas emissions must be reduced if climate and environment are to remain bearable. Page 713. See also 'Towards a history of the Sun', page 695.

Oncogenes' real role

The only cellular protein known to interact with the product of the c-*ras* proto-oncogene is 'GAP', which activates the GTPase activity of the protein $p21^{ras}$. Overexpression of GAP is now shown (page 754) to suppress transformation of cells by the normal c-*ras* product, suggesting that GAP is a negative regulator of $p21^{ras}$. On page 719 it is reported that stimulation of the antigen receptor of T lymphocytes causes a rapid activation of $p21^{ras}$. The mechanism seems to involve a decrease in GAP stimulation of protein kinase C. And see News and Views, page 696.

Life force

The theory that the formation of FeS_2 (pyrite) from FeS in an energy-producing reaction could have been a direct source of energy for early forms of life, receives support from the demonstration that the monosulphide can be oxidized in the presence of hydrogen sulphide under strictly anaerobic conditions. Page 742.

Sequence chance

A statistical analysis suggests that the property of proteins of having a unique stable fold may not be a rare characteristic selected by evolution, but actually be true of a high proportion of all possible sequences. Page 773.

Behind the lines

Two more unidentified spectral absorption bands have been added to the list of 80-plus such features that originate from unidentified atoms or molecules in the interstellar medium. The two new bands are in the infrared, one at a wavelength 1.5 times longer than previously observed, and place further limits on speculations as to what interstellar compounds are causing these mysterious lines. Page 729.

On guard cells

The use of 'caged' compounds that release calcium and inositol trisphosphate on photoactivation reveals ionic changes that could explain the change in guard cell turgor underlying stomatal closure. Pages 766, 769 and 700.

Collision course

Does the US suggestion of a $1.6 million contribution from Japan to the costs of the Superconducting Super Collider represent a worthwhile international cooperation? S. S. Yamamoto is not convinced. Commentary, page 692.

Cautious forecast

As climatologists refine the 'general circulation models' on which predictions of climatic response to greenhouse gases, deforestation and so on are based, more realistic treatments of the various components of the system are sought. On page 734 Pitman *et al.* warn that such models can be highly sensitive to the fine details of land-surface/atmosphere coupling.

Cancer model

Mice transgenic for the viral oncogene v-*jun* are phenotypically normal, but deep wounding leads to abnormal wound repair with frequent progression to fibrosarcomas and increased expression of the v-*jun* gene. Such mice could provide a good system to study the steps leading to cancer. Page 756.

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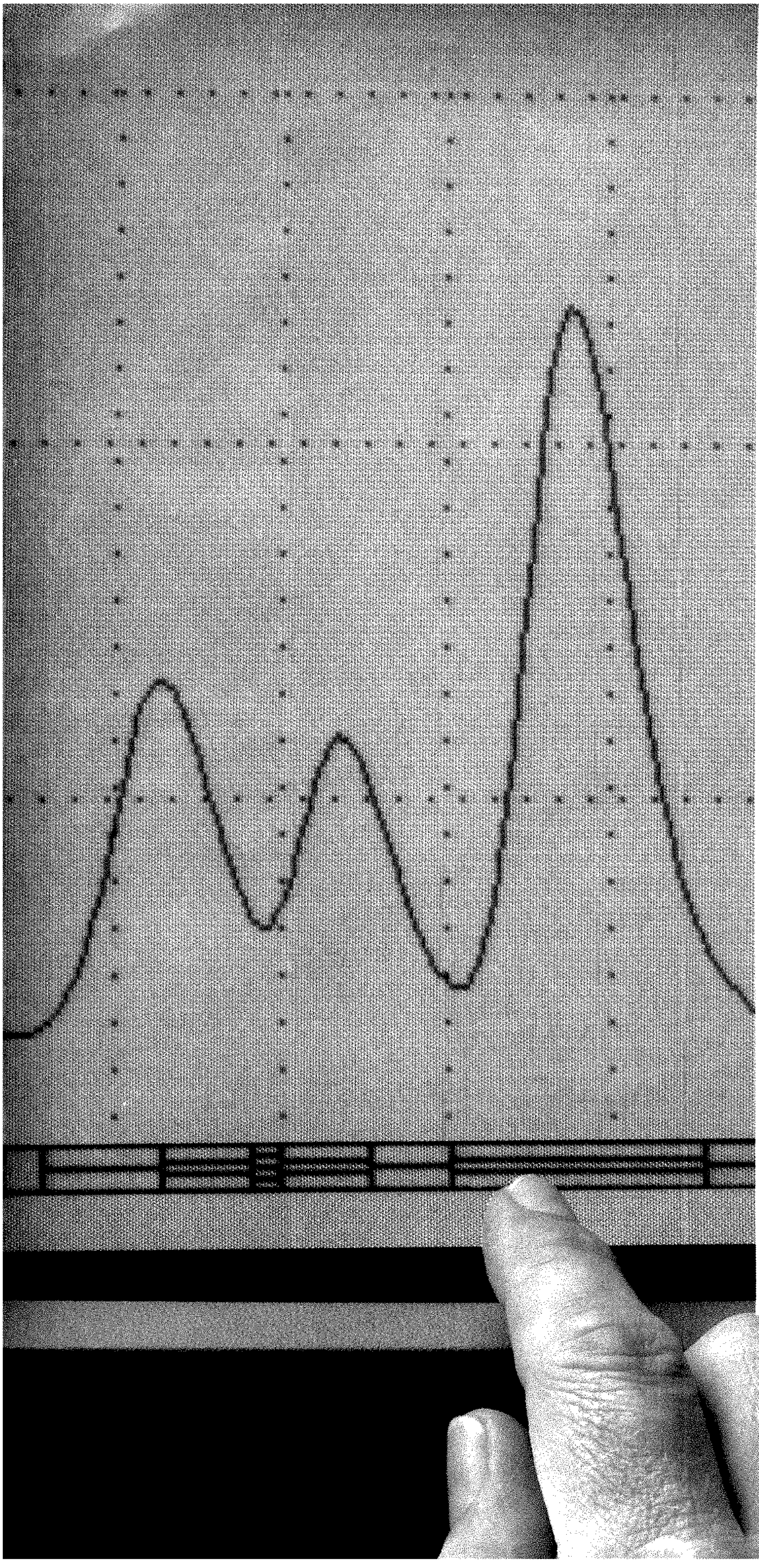

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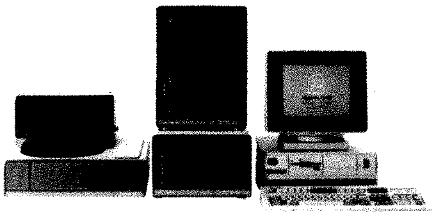

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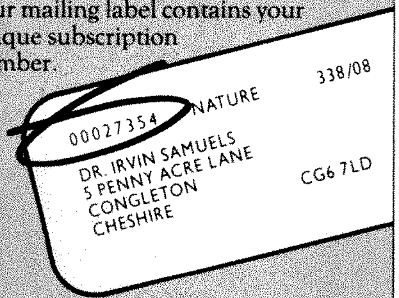

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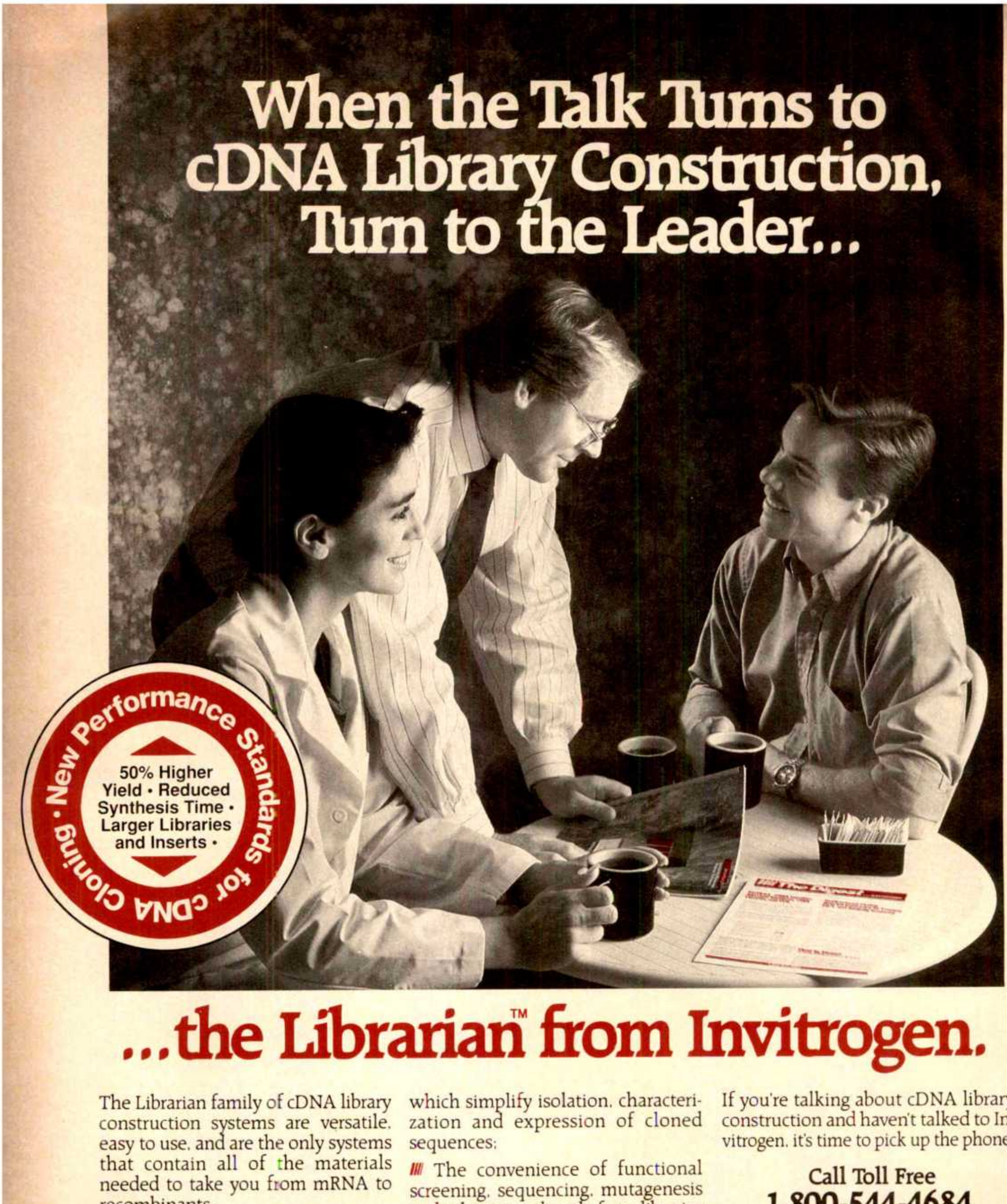
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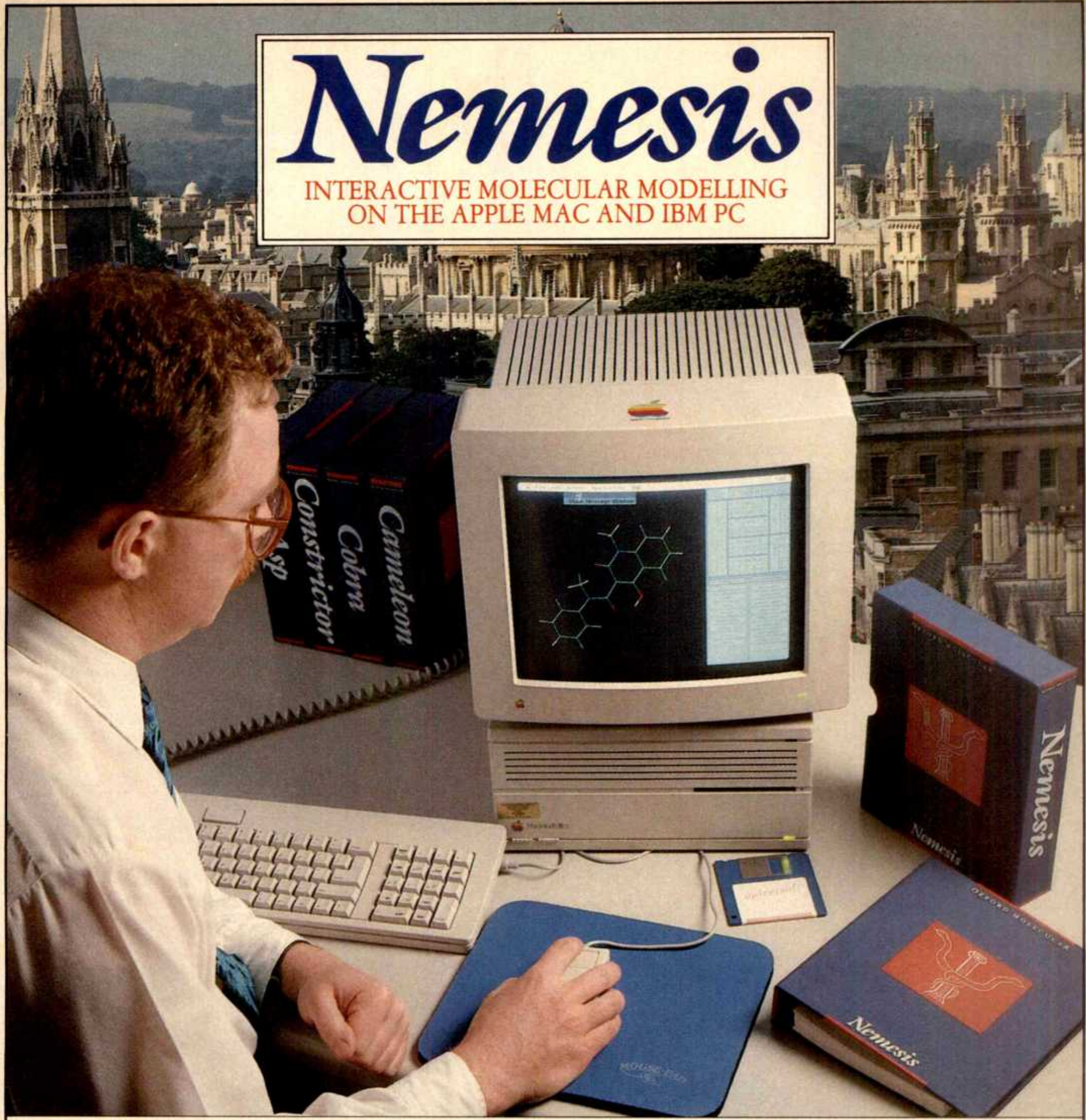

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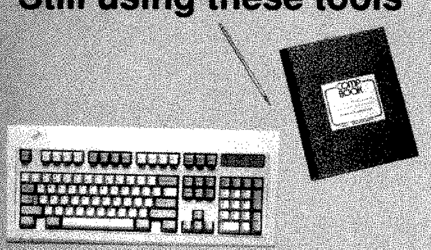

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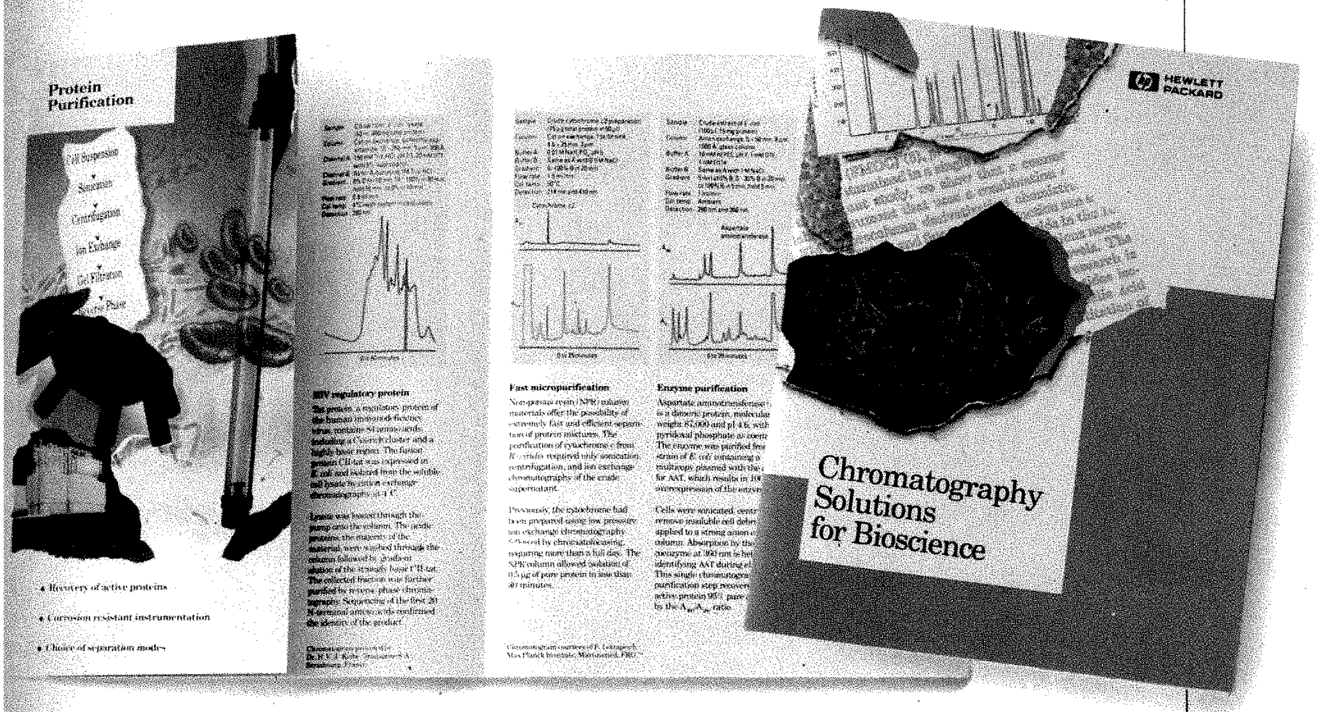

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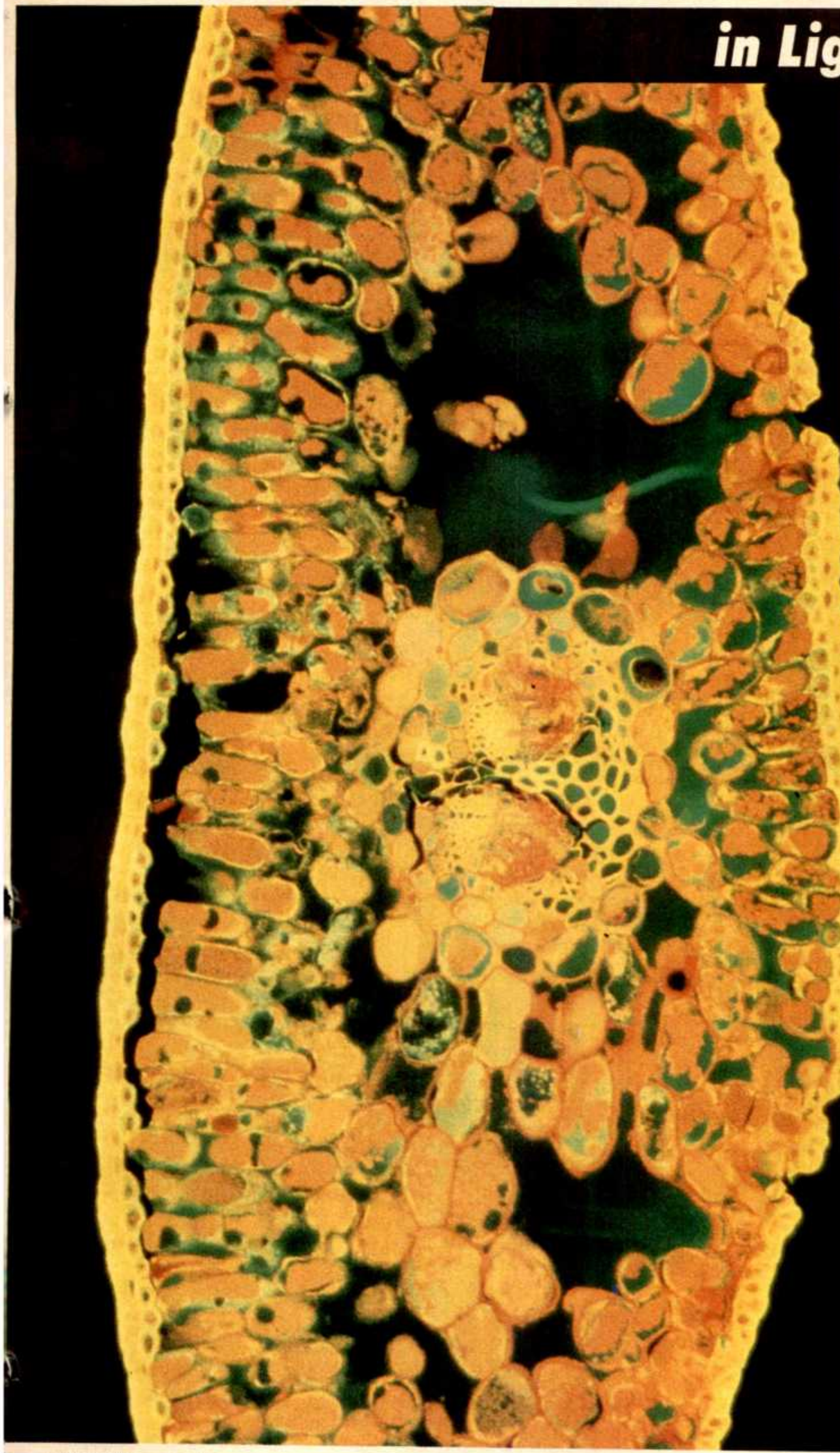

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Beyond the trouble in the Gulf

No amount of resolution by the United Nations can hide the truth that even a quick resolution of the problems created in Kuwait by Iraq (which seems unlikely) will not restore stability to the Middle East.

THE general consternation that the end of the 40-year Cold War should so swiftly be followed by the prospect of serious trouble in the Middle East is understandable, but this disconcerting turn of events is not surprising. Iraq, which annexed Kuwait at the beginning of the month, has only just reached an uneasy stalemate with its neighbour Iran after eight years of warfare that left its economy in ruins and few of its military objectives attained. The general indifference of governments elsewhere to that conflict, both middle-sized powers in the West (such as Britain and France) as well as the then two superpowers (the Soviet Union and the United States) is chiefly explicable by the way in which both combatants kept supplies of oil flowing. Each, after all, had to pay for its military equipment. Such interest as the Iran–Iraq war evoked resulted in mild support for Iraq, given the general distaste for the previous Khomeini regime in Iran. But now the shoe is on the other foot.

Iraq makes no secret of its awkwardness as an adversary. It is equipped with chemical weapons (poison gas) and has experience in their use, both in the war with Iran and against its own population of Kurds, in northern Iraq. It has a supply of long-range surface-to-surface rockets, but little experience of their use. Its flirtation with means for making a huge gun was fanciful, but has probably come to nothing. Iraq is not yet a nuclear power, and is unlikely ever to be one in its own right; one of the disturbing features of this month's developments is that the combination of Iraqi determination and Kuwaiti money may persuade some other states to join in such a venture. But the most menacing development of all is Iraq's apparent willingness to use foreigners caught by accident in its extended borders as hostages in future dealings with governments elsewhere. The unanimity of the UN Security Council in the days following the annexation of Kuwait remains the best hope that order will be restored in the Middle East by legal processes, not military conflict. But it remains to be seen whether the resolution of governments elsewhere will survive televised appeals for compromise by their trapped nationals.

That is one reason why attention should be given now to the causes, rather than the occasion, of the conflict in the Middle East. The underlying difficulty is the revenue from the sale of crude oil earned by states in the Middle East, and the fortuitously unequal distribution of that income. Iraq, with 15 per cent of the total production of the Organization of Oil Exporting Countries (OPEC), was not badly off, but its income from the sale of $20-a-barrel oil was not sufficient to pay for the war with Iran and is plainly insufficient to sustain its plans to be a substantial military force among Arab states (whence, of course, the move on Kuwait). But Iraq is at least distinguished from the other oil-producers of the Middle East by its plan for spending its oil revenues on a national programme of some kind, being militarily strong in this case. The bulk of what other oil-producing states earn is routinely lent back to the purchasers of oil, usually in the form of credits controlled by named individuals rather than by OPEC governments. The chief effect of 20 years of high-priced oil is that it has created many millionaires among the populations of the Middle East.

The conspicuous failure of the same 20 years has been the poverty of constructive development in the oil-producing states of the Middle East. It is true, of course, that the wealthier governments have set out to provide their people with advanced health services, usually relying on physicians and other paramedical workers from elsewhere to service them. There have also been developments in education, but they are mostly unremarkable. The oil-producers of the Middle East, blessed though they have been with income of the order of $200,000 million a year from oil, seem not to have turned their minds to creating a first-rate university somewhere. (The University of Cairo, in impoverished Egypt, remains the Arab world's outstanding university.) Similarly, there has been too little investment in industrial enterprises, even in the downstream processing of oil itself. The oil producers have also spent substantial parts of their funds on preparation to defend themselves and their fortunes; the experience of Kuwait shows how effective that has been. It is no wonder that Kuwait has fallen to Iraq.

There is no way in which the governments now joined in the enterprise of forcing Iraq to retreat from Kuwait can force more enlightened policies on the undemocratic governments elsewhere in the region, but they can at least say loudly and publicly what they think is wrong. Both the vulnerability of the Arab oil-producing states to attacks such as that of Iraq on Kuwait and to Shiite rebellion and revolution are simply consequences of their hierarchical social structure and nepotistic ways of sharing unimagined wealth. While these practices continue, troubles like that which has now arisen are bound to recur. □

Integration problems

Science has a fighting chance of survival in the eastern part of Germany. But will it seize the moment?

WHAT is to be done to integrate the research establishment of East Germany, hitherto supported from Berlin, with that of West Germany, supported from Bonn? It has been plain since the outset of the reunification project that there would be serious difficulties in absorbing East Germany's stalinist Academy of Sciences into the quite different pattern of research supported by, among others, the Deutsche Forschungsgemeinschaft (DFG) and the Max-Planck Gesellschaft (MPT), many of which were vividly made plain by the letter from Dr Werner Franke last week (*Nature* **346**, 603; 1990). Put bluntly, the problem in East Germany is that almost all research is supported through the academy and carried out by institutes whose tenured members conduct research which is often second-rate and whose personal reputations have been sullied by their association with the regime whose last vestiges will disappear with the elections planned for December. What will happen to the eastern academy institutes after that?

Wisely, the West German research minister, Heinz Riesenhuber, has agreed that 18 months should pass before the fate of the institutes and their researchers is finally decided. To have acted otherwise would have been to emulate old-fashioned East German heavy-handedness. The hope is that the interval will allow a sober evaluation of the parts of the old academy system worth saving. But the process is bound to leave scars.

So how should this breathing-space be spent? One possibility is that the academy system may be able to shrink itself to a more modest size. That is what Riesenhuber himself expects. The snag is that the end result would be a series of institutes not necessarily of a kind that could be integrated into the West German system. It would be much better that the interval should be spent in allowing elements of the research enterprise in the east, from individuals to whole institutes, who consider they have a legitimate claim on funds from the West should use the interval to apply for support from DFG, MPG or other western sources. That would also be a painful process, but one that people could understand.

However the integration is accomplished, it will not be free from recrimination. Under the old regime, the patronage of the stalinist government was a necessary requirement for even modest success in science. People at odds with the ideology were unable to meet their peers by travelling abroad, and were often denied facilities for research. To what extent, now, should there be positive discrimination in favour of these people? And may not those who sold their soul to the old regime also be seen as victims? That is a question that will keep the evaluators awake at nights, not only in East Germany but in the rest of reforming Eastern Europe. ☐

Untransparent grants

The British Medical Research Council will have to be more open about its research policy.

DURING the past ten hard years, the British research councils have miraculously managed to keep the respect of their constituents, researchers at British universities, by the transparent fairness of their decisions. Even when there has not been enough money to go around, grant applications have been scored with apparent objectivity by the peer-review system, and many applicants destined for disappointment have been told that their proposals would in normal times have been supported. But there are now signs that the strains on the system may be undermining it. The fuss about the refusal of the Medical Research Council (MRC) to renew a research grant at Cambridge (see opposite) is a sign that strains on the British research-support system are now insupportable.

The circumstances are not entirely clear, although it seems to have been determined that the ten-strong Medical Cryobiology Group (which costs £250,000 a year) is to be disbanded. The research bears on the physics and the physiology of the preservation of organs for transplantation in which Cambridge is a leading British centre. Referees (not exclusively from Britain) appear to have given the work at Cambridge glowing testimonials, but the MRC Cell Board seems to have offset these high opinions with the conclusion that the research, while interesting, lacks urgency and, indeed, the potential for making a mark on research internationally. At least some members of the subcommittee that reached that conclusion are now dissenting from the decision to close down the research group, saying that their opinions have been misrepresented. Transplant surgeons, in Britain and elsewhere, are meanwhile up in arms.

The issue of principle raised by these developments concerns the degree to which grant-making bodies such as the MRC must make their decisions intelligible to the wider world. Especially when research funds are as scarce as they have become in Britain, nobody expects that all decisions can be clear-cut, or determined exclusively by scientific quality. Questions of whether the research concerned can be afforded a place in some national pattern of activity are also legitimate and proper. What the MRC appears on this occasion not to have appreciated is that decisions based on these broader considerations must also be argued publicly if they are to make sense to those directly affected and to the research community in general. It will be disgraceful if the MRC has sought to avoid the difficulty of justifying an unwelcome decision by concealing its reasons for reaching it, which is the obvious inference of the Cell Board's report. In any case, the MRC has no choice but to argue the case for strategic decisions publicly. Or rather, if it chooses to act otherwise, it must recognize that it will lose the support of its research community. ☐

MRC's cryobiology closure evokes protests

■ Reviewers' recommendations over-ruled
■ Lack of clinical results cited

London

THE United Kingdom's Medical Research Council faced a storm of protest from British and international research communities last week over its decision to close a small cryobiology unit in Cambridge, the only group developing techniques for freezing human tissue and organs in Britain.

Condemned by leading biomedical researchers on both sides of the Atlantic and as far away as Australia, the decision will lead to the dispersal an internationally valued team of 10 researchers. All told, the group, led by David Pegg, currently receives £250,000 a year out of the MRC's budget of £180 million. At the heart of the furore over the closure is the accusation that the MRC wrongly over-ruled the opinion of a panel of expert referees who were unanimous in recommending continued funding of the unit. One of them, Professor Felix Franks of the University of Nottingham, has even denounced the MRC decision as "a corruption of the peer review system".

Appointed last year by the MRC to conduct a routine review of the Cryobiology Unit's work, the referees all submitted highly favourable reports. But earlier this year the council's Cell Board — one of several committees that are responsible for making funding decisions — acting on a report from its own subcommittee, gave the unit only a 'beta' rating, a verdict that, with many 'alpha'-rated projects currently failing to win funds, guaranteed the unit's closure.

The Cell Board judged the unit's work to fall below the "competitive standard" in the current financial climate, says Nick Winterton, head of the secretariat of the MRC. That judgement, while apparently at odds with the views of specialists, reflects the board's "wider scientific representation", he adds. Effectively, the unit's fate was sealed by a concluding remark in the subcommittee report that, despite 10 years of funding, clinically useful applications seem to be "still around the corner".

Be that as it may, the most outspoken critics of the decision are transplant surgeons and clinicians. In a letter of protest, Sir Roy Calne, Professor of Surgery at Cambridge, said he was "amazed" at the decision and that he "earnestly requested the council to reconsider". The MRC, says Calne, does not appreciate the enormity of the problem being tackled by the group — how to keep tissue at sub-zero temperatures without it being damaged by ice crystals — which is "probably as difficult a thing to crack as the immune system in organ rejection". At least 70 other clinicians and scientists have also lodged protests.

These might have proved easier for the MRC to deflect if the two cryobiologists invited to sit on the Cell Board's subcommittee had not already voiced dissent. One of them, Peter Mazur of Oak Ridge National Laboratory in Tennessee, complained to the Cell Board's chairman saying that his positive views had been ignored and describing the report's emphasis on "clinical isolation" as "a distortion". In a letter to Dai Rees, head of the MRC, Mazur said that he had been "thunderstruck" to hear of the recommedation to close the unit. According to Winterton, the original report was not amended but the reservations of the two dissenting cryobiologists were communicated to the Cell Board before it made its final recommendation.

For some critics, news that a medical transfusion department currently being set up in Cambridge University had intended to incorporate the cryobiology unit as its main research arm made the council's decision even harder to swallow. Such a move would have answered the chief criticism of clinical isolation, says Calne. And concern over the closure rose further still when it emerged that plans to establish a new bank for human heart valves in Cambridge had been shelved because of the MRC's decision.

The planned closure is assumed by many critics to reflect on the need for a strategic reorganization of funding within the MRC rather than the quality of the unit's work. But regardless of any protest, it is unlikely that the MRC's decision will be reversed — the Cell Board has already had ample opportunity to review its original decision in the light of representation, says Winterton.

David Concar

DRUG APPROVAL

Fast track for AIDS and cancer drugs

Washington

NEW drugs for life-threatening diseases such as cancer and AIDS should be handled more quickly by the US Food and Drug Administration (FDA), concludes a report released last week by the President's Cancer Panel. The recommendations come from a committee, chaired by Louis Lasagna of Tufts University, asked by the Cancer Panel to determine whether the approval criteria for cancer or AIDS drugs should be modified, and to identify barriers that have impeded the access of cancer and AIDS patients to new treatments.

The committee concurs with a recent Government Accounting Office opinion that the FDA is under-financed and undermanned. But a substantial increase in federal funds for FDA is unlikely in the present fiscal climate, and the committee recommends that the FDA should reduce its burden by relying more heavily on outside reviewers. Phase I and II investigational new drug (IND) applications that are filed by academic "non-commercial" researchers, many of which involve new uses for approved drugs, should be reviewed by Institutional Review Boards, the committee says. The report also suggests that drug companies should be given the option of paying "user fees" to the FDA for an expedited review of a drug by experts outside the FDA who have no conflict of interest.

To hasten the approval process for cancer and AIDS drugs, the committee urges the FDA to make its criteria more flexible: for example, in assessing the therapeutic benefit of a drug for the treatment of life-threatening condition, proof that the drug will prolong life need not be a prerequisite for FDA approval. Instead, The report suggests that a drug that produces tumour regression in more than 20 to 30 percent of patients who have not responded to alternative therapies should be approved.

Similarly, the development of AIDS drugs could be facilitated by the approval of drugs that not only cause a rise in the T4 cell count but also improve a patient's quality of life.

The committee also believes that phase III cancer studies, designed to compare the efficacy of an experimental drug with that of a marketed drug, can delay the drug approval process and should not be a requirement for marketing approval.

The committee supports the rights of patients with life-threatening diseases to obtain accelerated access to experimental drugs, and although Lasagna accepts that making new drugs available for marketing at an earlier stage may increase the dangers of toxicity, he believes that "more good than harm" would come from implementing the committee's recommendations. The committee points out that patients with life-threatening conditions, for whom there are no alternative treatments, are often willing to accept the greater risks associated with new therapies.

Diane Gershon

HUBBLE SPACE TELESCOPE

Radio to the rescue

Washington

SIMILARITY between the distorted optical images produced by the out-of-focus Hubble Space Telescope (HST) and the signals normally generated by multi-element radioastronomy telescopes will allow some parts of the HST's planned observing programme to be salvaged, astronomers said last week. Image processing techniques developed over the past decade by the National Radio Astronomical Observatory (NRAO) have proved surprisingly effective in filtering out the blurry 'haloes' that are the principal symptom of the HST's malaise.

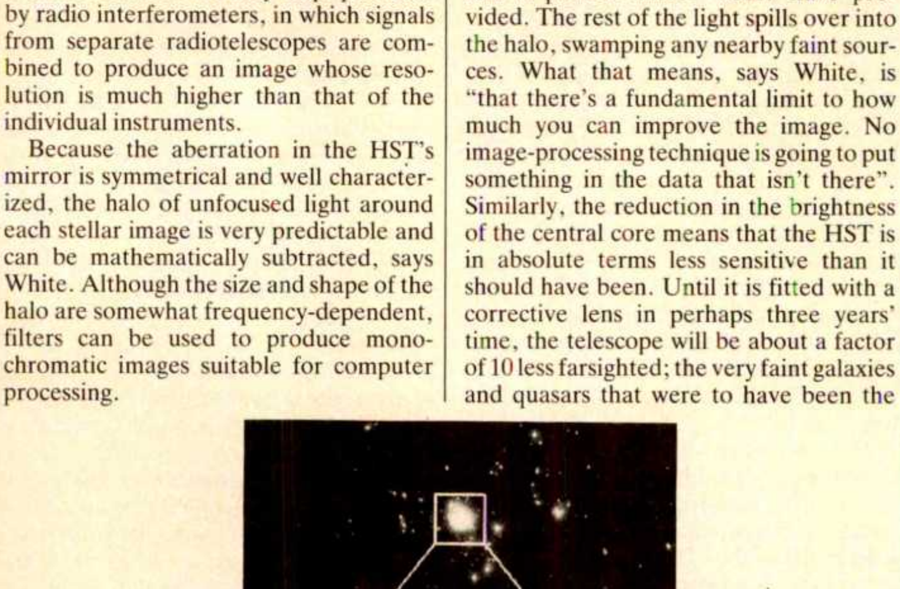
Rick White, of the Space Telescope Science Institute in Baltimore, says that after HST's problem was announced, "we noticed right away that the fuzzy images, with a well-defined core and nasty side lobes, looked just like radioastronomy data". The HST images, produced by an optical system whose primary mirror suffers from spherical aberration, have a sharp central core surrounded by a diffuse halo of improperly focused light (see *Nature* **346**, 3; 1990). This image structure is characteristic of intensity maps produced by radio interferometers, in which signals from separate radiotelescopes are combined to produce an image whose resolution is much higher than that of the individual instruments.

Because the aberration in the HST's mirror is symmetrical and well characterized, the halo of unfocused light around each stellar image is very predictable and can be mathematically subtracted, says White. Although the size and shape of the halo are somewhat frequency-dependent, filters can be used to produce monochromatic images suitable for computer processing.

Photographs released last week by the National Aeronautics and Space Administration (NASA) show how image processing resolves individual stars within the star cluster R136, part of the 30 Doradus nebula (see below). The upper image was made with the Wide Field/Planetary Camera on 3 August; the unprocessed enlargement at lower right shows about 60 stars, more than can be seen from the ground, but in the image at lower left, enhanced using computer codes derived from the NRAO algorithms, hundreds of stars stand out. Beyond its technical significance, this image represents a genuine scientific achievement — the measured density of this cluster of young, massive stars is higher than anything previously seen.

Although image processing will restore much of the HST's full resolution, according to White, the dynamic range (the ability to detect faint objects when they are close to bright ones) cannot be so easily restored. In HST's blurred images, only 15 per cent of the total light is in a central core, rather than the 70 per cent that a perfect mirror would have provided. The rest of the light spills over into the halo, swamping any nearby faint sources. What that means, says White, is "that there's a fundamental limit to how much you can improve the image. No image-processing technique is going to put something in the data that isn't there". Similarly, the reduction in the brightness of the central core means that the HST is in absolute terms less sensitive than it should have been. Until it is fitted with a corrective lens in perhaps three years' time, the telescope will be about a factor of 10 less farsighted; the very faint galaxies and quasars that were to have been the HST's most distant goal will remain hidden until then. Nevertheless, if observation periods are extended and the new image-processing techniques used, White thinks that as much as 80 per cent of the science planned for the HST's first two years appears to be back within reach.

Christopher Anderson



Radioastronomy techniques succeeded in teasing out the information from Hubble's original effort. The merits of a partially functioning Hubble are the subject of a letter in the Correspondence pages.

■ A statement released last week by Lew Allen, chairman of the HST Board of Investigation, confirmed that the entire half-wavelength error in the primary mirror's shape could be attributed to a 1.3-mm positional error in one of the components of the reflective null corrector (see *Nature* **346**, 599; 1990).

Tantalizingly, Allen observes that some of the spacing washers in the null corrector have a thickness of 50 thousandths of an inch — or 1.3 mm. A misplaced washer may turn out to be the ten-cent cause of a billion-dollar mistake. **D.L.**

LARGEST TELESCOPE

Go-ahead for Japanese telescope?

Tokyo

JAPAN'S Ministry of Education, Science and Culture (MESC) has at last decided to start building the world's largest telescope, according to a leak in the Japanese press on Monday.

Plans for the 7.5-metre single-mirror telescope, to be built in Hawaii, have been in the works for years (see *Nature* 311, 5; 1984), and this fiscal year MESC acquired 13 million yen ($90,000) in 'preparatory' funds for the project. But MESC officials have insisted that a final go-ahead for the project, which is eventually expected to cost 45,000 million yen ($300 million), has not yet been given (*Nature* 343, 295; 1990).

According to a report in the Yomiuri newspaper on Monday, MESC will apply at the end of this month for 600 million yen ($4 million) to begin construction of the telescope in fiscal 1991.

Keiichi Kodaira of the National Astronomical Observatory, a well known promoter of the project, could not confirm the Yomiuri report on Monday. He says a final decision is expected on August 22 or 23 and will be officially announced at the beginning of September. But he says he has the impression MESC is "very positive".

The telescope has competition within MESC from another large project in the form of Superkamiokande, a proposal from Tokyo University for the world's largest observatory for neutrinos and proton decay (*Nature* 331, 379; 1988). Supporters of that project are also fairly confident of winning MESC funds to commence construction next year, but they too are waiting for an official announcement at the end of this month.

David Swinbanks

TELECONFERENCING

Ready for the future?

Pullman, Washington

LAST week, 3,000 agricultural scientists around the world saw the future, and apart from a few technical glitches, dead spots and occasional lack of focus, it worked. An international symposium on plant biotechnology put on by Washington State University was the first interactive televised scientific conference to be broadcast worldwide, and although it was not without hitches it showed that that satellite networks are finally up to the task of video conferencing.

The symposium, a year in planning, illustrated strengths and weaknesses of teleconferencing technology. With an audience distributed at 70 viewing centres worldwide, it brought together plant biotechnology experts with scientists in developing countries who might not have been able to attend an international meeting. Conference officials estimate that at about half of the viewing centres, local organizers used the three-hour symposium as the nucleus of longer local meeting. Observers at all sites were able to fax written questions during the conference to Pullman; by mid-point, over a hundred questions had been received. Several dozen questions were answered on-air, and the others received written responses.

The conference featured two panels of plant biotechnologists, one group in Pullman, the other at the Max-Planck Institute for Genetics Research in Cologne, West Germany. Pullman's five-member panel was composed of scientists from the United States, Mexico and Australia, along with an American journalist — Jack Reynolds of the United States Information Agency — who served as moderator. In Cologne, Jozef St. Schell, director of the Max-Planck Institute, led a four-person panel that included former International Rice Institute director M. S. Swaminathan and Konstantin Skyrabin of Moscow State University.

Except for a brief loss of audio from Cologne and some minor production glitches, the conference was technically flawless.

Much of the success was due to redundancy in the form of duplicate equipment at every stage of link (which included seven satellites and an equal number of ground stations), and a large production crew of professionals and students from Washington State University's telecommunications programme.

But as technically impressive as the symposium undeniably was, it illustrated some of the traditional problems of international conferences, as well as some new ones. With an audience of biotechnology researchers, Third World agricultural scientists, students, and even Washington State legislators, the conference oscillated wildly in tone, technical content, and direction.

A basic 15-minute taped primer on genetic engineering by Schell was immediately followed by a debate on the fine points of RFLP (no definition given) techniques by other panel members. Summaries given at the end of each one-hour segment appeared to have been largely prepared beforehand, reflecting little of the unscripted debate. And a prepared presentation on the application of biotechnology to world food production by Robert Fraley of the Monsanto Corporation (one of the conference sponsors) came dangerously close to becoming a video advertisement for Monsanto products.

The basic problem, however, appeared to be that because little more than a 5-metre satellite dish and a television set was needed to plug in to the conference, the audience became impossibly broad, ranging from leading researchers to interested but nonscientific members of the public. The organizers tried to include a little something for everyone, with the result that few participants seemed to find the conference very satisfying. An exit survey taken at the Pullman site showed that the symposium did a poor job of demonstrating research techniques, conveying useful scientific information or providing new research ideas.

On the other hand, most participants gave the conference high marks for providing access to scientists who would not otherwise have been available. And many said that the conference had been their first introduction to the concerns of the Third World — the issues of access to research, costs, and preservation of traditional farming techniques. In one exchange that clearly illustrated the debate within the developing world on the hazards of biotechnology, Luis Herrera-Estrella of the Mexican Centre for Research and Advanced Studies (and a member of the Pullman panel) said that Mexico "is most interested in food production. The ecological impact [of biotechnology] is less important". But Indian scientist Swaminathan, speaking from Cologne, quickly jumped in to warn against the use of the Third World as a biotechnology laboratory. "I disagree that it does not matter as much in the developing world. Ecological groundrules must be the same wherever they are on the planet", he said.

Although the jury is still out on the scientific success of teleconferences, the symposium showed that electronic meetings can be substantially cheaper than their traditional equivalents. Total costs amounted to about $300,000, the majority of which was covered by in-kind donations from a half-dozen telecommunication companies. A traditional conference with the same attendance would typically cost the organizers about $1 million and the participants another $3 million for travel and lodging, according to Washington State University administrator Donald Hanna, who co-chaired the event.

But the larger lesson, he said, is that successful global teleconferences will have to cover fields that are changing too fast for the scientific journals to keep up with, stick to subjects like plant biotechnology and involve issues of worldwide importance, such as health, food, or population.

Christopher Anderson

AIDS TESTING

Trial approved for saliva test

San Francisco

A NOVEL saliva-based test for HIV (human immunodeficiency virus) infection, which may ultimately lead to a 'do-it-yourself' AIDS test, has been approved by the US Food and Drug Administration (FDA) for human clinical trials. The technique, developed by Epitope Inc. of Beaverton, Oregon, may make AIDS testing simpler and more widely available because antibodies are collected from the mouth without the need to draw a blood sample.

In the test, a treated pad is placed between the gums and cheek for two minutes to collect antibodies to HIV, which are present in the saliva. The pad is then placed in a vial and mailed to a laboratory where it is submitted to the standard HIV test now used on blood samples. In pre-clinical trials on 600 patients, Epitope scientists report that the collection device has been perfectly reliable, leading them to believe the test will be "fully equivalent" to blood tests.

FDA approval will allow Epitope to carry out clinical trials for nine months at five sites nationwide, where matched samples of blood and saliva will be compared. If marketing approval is then granted, the company plans to make the device available worldwide. Potential users include police and emergency medical personnel who request frequent testing for HIV, and developing countries where blood testing may be impractical.

The company is now considering ways to address questions of counselling and anonymity that would arise with widespread use of the test. One option under consideration is that AIDS hotlines or counselling centres would be contracted to inform users of the results of their tests, and of what can be done following a positive result. Epitope hopes ultimately to produce an AIDS test, based on the saliva collection device, which could be conducted entirely at home.

Elizabeth Schaefer

NUCLEAR RESEARCH

Fallout hits research

Munich

OPPOSITION to nuclear power, a cause embraced by citizens' initiatives and splinter parties a decade ago, has begun to take over the political mainstream in West Germany, with potentially dire consequences for scientific research. In a decision that is certain to have broader implications, West Berlin Mayor Walter Momper, a Social Democrat, last week surprisingly withdrew his support for a 10-MW research reactor located at the Hahn-Meitner Institute (HMI). The move endangers the existence of HMI, one of 13 national laboratories in West Germany.

Momper had originally promised to award a licence to the refurbished reactor by 31 July at the latest. But the environment minister in his coalition government, Michaele Schreyer of the *Alternative Liste*, refused to issue the licence because of the lack of permanent storage space for the radioactive waste produced by the reactor (see *Nature* **346**, 405; 1990.) *Alternative Liste* is the local branch of the environmentalist Green Party.

After a majority vote on 7 August by the West Berlin Senate in favour of licensing the reactor, researchers had been expecting Momper to reverse Schreyer's decision somehow, for example by removing her jurisdiction over licensing. But such a move would have endangered the fragile West Berlin coalition, and elections are only three months away. Instead, Momper returned from holidays on 13 August with the surprising news that he had been at least partly convinced by Schreyer's arguments.

Momper then offered HMI two unappetizing options. If he found Schreyer's case against licensing the reactor "watertight", he said, there would be nothing more he could do, and it would be up to HMI to bring a lawsuit against Schreyer. If not, he would reopen the licensing procedure, which would probably require new public hearings and could not be concluded until after the forth-coming elections. In either case, it would take months, if not years, for the reactor to receive a licence.

The overhaul and upgrading of the reactor from 5 to 10 MW was completed last year at a cost of DM170 million (about $110 million). Most of the money came from the Research Ministry in Bonn, which pays 90 per cent of the operating costs of the national laboratories (*Grossforschungseinrichtungen*). The reactor is to be used as a neutron source for biology, medicine and chemistry as well as physics.

Tired of broken promises from Momper, West German Research Minister Heinz Riesenhuber (Christian Democrat) last week issued an ultimatum: license the reactor by 21 August, or Bonn will pull out of financing HMI. According to West Berlin Senate spokesman Werner Kolhoff, West Berlin is already overburdened by the costs of reunification and could not afford the DM100 million a year that would be required to keep HMI open if Bonn pulls out.

Some see Momper's and Riesenhuber's actions as gestures made solely to avoid losing face. As a prominent representative of a party that is officially against nuclear energy, Momper cannot afford to back the HMI reactor unless final waste storage is secured. And Riesenhuber, who has been threatening to pull out of HMI for months, cannot easily back down now.

On this view, HMI will have to be patient for a few months until all-German and all-Berlin elections, both of which are scheduled for 2 December. After that, perhaps, the federal government will be able to force the licensing of the reactor in the case of a legal challenge, a possibility that comes about because of the murky legal status of West Berlin. For at least a few more weeks, it will remain under the jurisdiction of Allied occupying forces, not of Bonn.

But the patience of HMI is nearly at an end. Spokesman Thomas Robertson called the situation at HMI "miserable". HMI has lost one candidate for the open position of director and may well lose a second in the next few days. Researchers in unrelated fields such as solar cell development who are contemplating moving to HMI are bound to be influenced by the worries about the institute's future, says Robertson.

The turmoil at HMI occurs against a backdrop of rising opposition to nuclear power in West Germany. The Social Democrats (SPD), one of the two largest political parties , dropped their support of nuclear power in 1987 following the Chernobyl accident. SPD has won a series of elections in the *Länder* (states) which have given it a majority in the Bundesrat, the upper house of parliament. A victory by SPD in forth-coming national elections could turn the West German nuclear industry on its head, with an equally devastating if narrower impact on science. The problem may become especially acute at Garching, near Munich, where the Bavarian government has hesitated to replace an ageing research reactor at the Technical University of Munich because it feared a local backlash.

The dispute about the HMI reactor has already endangered Berlin's standing as an attractive site for research and industry. Both the heads of West German research organizations and the Chamber of Commerce and Industry of West Berlin have issued messages to that effect in recent weeks. **Steven Dickman**

TECHNOLOGY RESEARCH

MITI heads fo inner space

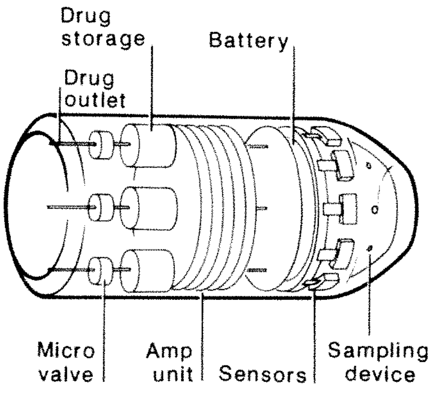
Tokyo

JAPAN's ever-ambitious Ministry of International Trade and Industry (MITI) seems to have found in Steven Spielberg's film *Inner Space*, about a microsubmarine that ventures through human blood vessels, an idea for its next venture. At the end of this month, MITI's Agency of Industrial Science and Technology (AIST) will submit a budget request for ¥30 million ($200,000) to launch a 'microrobot' project next year, with the aim of developing tiny robots for the internal medical treatment and repair of human beings. The robots will also be used for the maintenance and repair of nuclear power plants and may find applications in space.

Although the initial budget request is small, as is usually the case with MITI projects in their first year, MITI is planning to pour ¥25,000 million ($170 million) into the microrobot project over the next ten years under AIST's 'large-scale' (Ogata) industrial research programme.

The Ogata projects are among the biggest in AIST's repertoire, and there was stiff internal competition within MITI for selection of the one new project that can start next year. Furthermore, the proposed budget and project duration are unusually ambitious — Ogata projects typically run for seven years on budgets of about ¥15,000 million.

Kenzo Inagaki, deputy director of MITI's industrial machinery division, which helped to formulate the project, says that although MITI's draft proposal discusses microrobots manipulating cells, robots that travel through blood vessels *à la* Spielberg are "at least a hundred years off". But he thinks that robots of about a centimetre in size that can enter the stomach and intestine are feasible.



Getting smart: a sketch of MITI's projected 'pill'. No delivery dates have yet been announced

One idea is to develop a 'smart pill' that will be equipped with a sampling device and sensors in the nose cone (to detect, for example, pH and temperature), and a drug delivery system in the rear.

Several robot manufacturers, including FANUC and Fuji Electric Co., are expected to join the project, together with semiconductor manufacturers such as Hitachi, NEC and Toshiba. And Inagaki says that, although "officially" Ogata projects are totally financed by MITI, industry is expected to contribute "an additional 30 per cent or so".

The project was proposed by a MITI committee of industrialists and academics headed by Naomasa Nakajima of the department of mechanical engineering at Tokyo University. Another member of the committee is Isao Karube of the Research Center for Advanced Science and Technology of the same university, well known for his work on biosensors, such as blood glucose analysers.

Inagaki says that research in the field of micromachines is only about five years old and that the number of researchers worldwide with expertise is very limited. For this reason, MITI officials are hoping to make the project into a joint international effort. But they do not intend to approach potential foreign collaborators until details of the project are finalized.

The project is an amalgamation of proposals and includes plans to develop microrobots for the maintenance and repair of nuclear power plants, chemical plants, gas and water pipes and aircraft engines. And MITI foresees a day when microrobots will be able to patrol the piping of nuclear power plants and carry out repairs "whenever an abnormality is detected", avoiding expensive shut-downs for maintenance and repair.

One likely addition to the project is the development of robots for use in space, already being investigated under another Ogata project.

MITI's budget proposal will no doubt be trimmed down in negotiations with the Ministry of Finance. And it remains to be seen if the Ministry of Health and Welfare will remain silent while MITI launches a project that delves deeply into the realms of medicine and drugs. But MITI will probably disguise and alter the project in such a way as to avoid objections.

David Swinbanks

CHEMICAL WEAPONS

Heated argument in prospect

Sydney & Boston

CRITICISM of the US Army's programme to incinerate chemical weapons on Johnston Island in the Pacific has come from several quarters in recent weeks, even though the incineration programme is already fully operational and more US chemical weapons are on their way to the island from bases in Germany. The debate, both in the United States and in the South Pacific countries, has caused a split between environmentalists and arms control advocates, but now seems unlikely to affect the fate of the Johnston Island programme. Nevertheless, it may well have an impact on the long-term prospects for incineration as a way for the superpowers to dispose of their vast and ageing stockpiles of chemical weapons.

At a meeting of the South Pacific Forum, held earlier this month in the small Pacific nation of Vanuatu, some participants continued to criticize the US chemical weapons incineration plant on Johnston Island as "another example of the Pacific being used by major weapons-producing states as an experimental area". Of the forum nations, only Australia and New Zealand supported the Johnston Atoll Chemical Agents Disposal System (JACADS), which began operation on 30 June (see *Nature* **346**, 5; 5 July 1990). A communique released by the island nations opposing the plant complained about the "significant uncertainties and risks" posed by the project.

Nor were the forum participants reassured by three scientific reports presented by Australia's Prime Minister, Bob Hawke, in support of US claims that the weapons destruction methods include adequate environmental safeguards. According to the US Department of Defense, workers at the JACADS incineration facility have already safely destroyed more than 800 artillery shells and some 2 tons of nerve agent. By now, Johnston Island, 1300 km southwest of Hawaii, has accumulated some 300,000 weapons ready for incineration, including mustard and nerve gas shells. Another 100,000 US shells consisting of 400 tons of Sarin and VX nerve gas are in transit from Clausen, West Germany.

The environmental advocacy group Greenpeace continues to be one of the most vocal opponents of JACADS, claiming that the high-temperature incineration process produces toxic bioaccumulative chemicals such as polychlorinated dioxins and furans. According to Paul Johnston, Greenpeace International spokesman on chemical weapons, there are four safer alternatives: chemical neutralization; photodegradation; electrochemical procedures and biodegradation.

These methods, Greenpeace argues, have the advantage over incineration that they would be done in closed systems, and would not "result in huge volumes of gases being pumped into the atmosphere". A recent Greenpeace technical review* of the JACADS programme reviewed the prospects and current status of each of these alternative methods. Johnston says that these alternatives could be developed into working techniques within 18 months if the US Army were to conduct accelerated research on them.

But Trevor Findlay, senior research fellow at the Peace Research Centre of the Australian National University in Canberra, does not believe that the alternatives offered by Greenpeace are totally safe either. As with incineration, Findlay points out, all four proposed alternatives require the lethal chemical agents to be taken out of their shells. The possibility of spills and accidents, he says, poses a safety and environmental threat from almost any proposed method.

Now that US Defense Secretary Dick Cheney and congressional committees have agreed in principle to cancel the US binary chemical weapons modernization programme, arms control advocates in the United States are heartened by US actions and see JACADS as "a big step forward" in the effort to live up to an agreement, reached with the Soviet Union last spring, that at least 80 per cent of both superpowers' chemical stockpiles should be destroyed. Many other countries, even some Pacific nations, have supported the demilitarization effort despite the potential environmental threat posed by chemical weapon destruction.

Australia, for instance, has supported JACADS as part of a push towards an international chemical weapons treaty (*Nature* **341**, 271; 1989). Unless it destroys its own stockpile, the United States is seen to be in no position to push other countries into signing a treaty.

But, environmentalists point out, even with the addition of the US chemical weapons from Germany, only some 7 per cent of the US stockpile will be destroyed at the Johnston Island facility. The Soviet Union's stockpile, none of which has yet been destroyed, is believed to be larger still. Thus the fate of most of the superpowers' chemical arsenals remains uncertain, and the environmental stakes are high. The Soviet's plans are unclear, although they too are working on incineration methods and are committed to collaborating with the United States in the weapons' destruction.

The remainder of the US chemical weapons arsenal is marked for incineration at eight domestic facilities, yet to be built.

Environmentalists say that they will fight the plan and push for more research into alternative methods. "Our fight at Johnston Island is not over yet and our serious concerns continue about the environmental damage caused by incineration", says US Greenpeace spokesperson Sebia Hawkins; "the larger fight in the United States is just beginning".

Tania Ewing & Seth Shulman

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* Greenpeace Review of Johnston Atoll Chemical Agent Disposal System (JACADS), July 9, 1990.

ARCTIC ENVIRONMENT

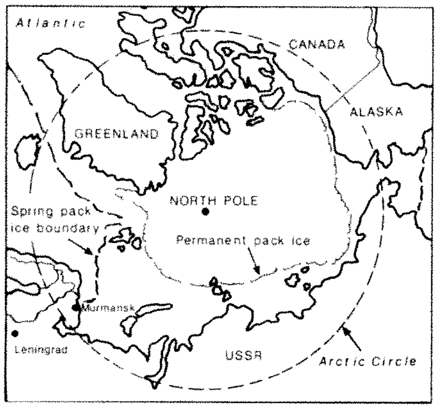
Soviets break the ice

Munich

PERESTROIKA and concern over global warming may combine to give Western researchers access to the Arctic through one of the most inhospitable ocean routes in the world. Taking a cue from a speech by Mikhail Gorbachev at Murmansk in 1987, the Soviet government earlier this summer commissioned an international feasibility study to determine the economic and ecological consequences of the year-round opening of the ice-choked passage (see map) known as the Northern Sea Route. Researchers from three Western institutes met at Oslo in late June to begin the study, which they expect to complete by March next year.

Controversial studies of global warming done by Peter Wadhams, a University of Cambridge researcher, and others, indicate that the sea ice in the Arctic regions may be thinning (see *Nature* **345**, 762 & 795; 1990). Researchers are eager to obtain data concerning ice thickness, ice motion, and climate history, all of which might yield valuable clues about global warming, and it seems certain that access to the Soviet Arctic will increase.

"Soviet [polar] scientists have always been enthusiastic about cooperating with the West", says Wadhams. "Now it has become official government policy". Collaborations were in the past always severely limited by Soviet security concerns, he adds.



As part of a joint West German-Norwegian-European Communities project, Norway recently received permission to place in Soviet waters a sonar device that measures the thickness of sea ice, says Wadhams. More such devices might soon follow.

For the Soviet government, the Northern Sea Route is of considerable economic importance. During each summer, when icebreakers are used to clear a path, 1 to 4 million tonnes of cargo are shipped through the passage, which provides a short route from the Atlantic to the Pacific. The distance from Hamburg to Yokohama is reduced from about 12,000 nautical miles (through the Suez Canal) to less than 7,000 nautical miles and the journey time from 30 to 22 days.

The Soviets have built a large fleet of nuclear-powered and conventional ice breakers, says Arnfinn Jørgensen-Dahl of the Fridtjof Nansen Institute in Oslo, and by using them to keep the passage open all year round they could help their own economy and earn hard currency by charging foreign ships a fee for the journey.

But there is concern among Western researchers about the possible danger to the environment of the Soviet project. "We assume there are dangers, especially from an oil spill", says Jørgensen-Dahl. Even though it could not stop the Soviet Union from opening the passage, the West can make a "positive contribution", he says, by ensuring that environmental damage is limited.

According to Wadhams, however, the dangers are "not negligible but not extremely serious". He does not expect oil tankers to use the passage. The environmental impact of opening the passage to international traffic, he says, would not be "any more severe" than the impact of current Soviet shipping.

Steven Dickman

ECOLOGICAL SCIENCE

Return of life unobserved

Mount St Helens, Washington

TEN years after the 50-megaton volcanic eruption that scorched square miles of woodland into Mars-like desolation, Mount St Helens is coming back to life, largely unobserved. Most of the scientists who raced here in the summer of 1980 to record the first glimpses of life in this barren world are back at their universities, unable to get further funding for research at the volcano, and their minutely compiled data from the early years after the eruption now gather dust. But researchers say that, ten years on, Mount St Helens is perhaps more interesting than it was immediately after the explosion.

Its original ecosystem removed at a stroke, conditions at Mount St Helens approach laboratory purity. The species that first repopulated the blighted landscape in isolated pockets are now beginning to interact, giving a unique perspective on survival and evolutionary strategies. In the hot springs that flow from the crater itself are thermophilic bacteria that may resemble the first life on earth. And in the volcanic ash and mud, 200 metres deep in places, small patches of plant life constitute a textbook case of succession, one species paving the way for the next in a process that can take centuries elsewhere.

But as the attention of funding agencies has moved elsewhere, unique scientific opportunities at Mt St Helens are being lost. "It's a fleeting opportunity", says Robert Williams of the US Department of Agriculture. "Things aren't static, they won't be there for us next year". During the first four years after the explosion the recovery of Mt St Helens was documented in detail, but those baseline studies have been abandoned and the continued biological evolution, now proceeding at exponential pace, is being recorded in only anecdotal fashion.

Research funding, which between 1980 and 1984 was over $1 million a year, is now less than $100,000 annually. More than 100 scientists studied the region in the early years, but now fewer than a dozen biologists make occasional trips — often with time and money borrowed from other research projects. Although 25 scientists from the US Geological Survey still monitor the volcano for seismic activity, research on accelerated erosion in the valley created by the vast 1980 mudslides is now better supported by the local logging company than by federal agencies.

Frustrated by the loss of a scientific opportunity at Mt St Helens, a group of biologists, geologists and ecologists earlier this year proposed a new "Mt St Helens Initiative", a $900,000-a-year scientific return to the region. The scientists hope to get money for their project next year.

But going from concept to an actual appropriation will take some salesmanship. For the Mt St Helens scientists, that may be the biggest challenge of all. Unlike physicists and astronomers, who have been forced to master the ways of Washington to gain funding for their expensive projects, the initiative's supporters, like many "small science" researchers, appear to be badly out of touch with the politics of science.

At a day-long helicopter tour of the volcano for congressional staff and journalists last week, the researchers fumbled their chance to make a direct appeal. Abstractions like "interdisciplinary studies across the disturbance gradient", "infrastructure" and "synthesis" may be full of appeal to environmental researchers, but the congressional staff were clearly lost. Acknowledging a tactical error, the researchers privately said that they would attempt to draft a more lucid proposal and send it to selected members of Congress within weeks. Given the modest request, some increased support seems likely. But how much more could be won with a clever pitch may remain a hypothetical question.

Christopher Anderson

BRITISH ASSOCIATION

A matter of education

Swansea

BRITAIN is "in danger of becoming one of the least adequately educated of all the advanced nations", according to Sir Claus Moser, president of the British Association (BA). In his address to the BA's 1990 meeting in Swansea earlier this week, Moser called for an urgent review of the whole of UK education and training, with the 1990s designated the 'decade for education'.

Moser welcomed the plan of Education Secretary John MacGregor to double UK participation in higher education over the next 25 years, noting that only 15 per cent of British school leavers now go on to higher education, compared with about half in the United States. But the expansion will need increased finance, Moser said, "of which so far there are no signs". Expansion of higher education must go hand-in-hand with a broadening of courses and the encouragement of under-represented groups such as women, ethnic minorities and the poorer section of the community, he added.

Moser's views are unlikely to be received favourably by a Conservative government determined to let market forces take the lead in educational policy.

Higher education reforms are a small part of a wide-ranging review envisaged by Moser. The fact that most children leave school at the age of 16 is "the most unacceptable feature in [British] education", he said. Britain should adopt a system similar to the French *Baccalauréat* (a broad-based university entrance certificate), he urged. Moser also voiced concern over the quality of UK secondary school science teaching — a recent BA-sponsored survey found that one in five British science teachers believe they lack adequate knowledge to teach their subjects. Regular 'refresher courses' are needed to improve this situation, Moser said, with salary increases to boost morale and recruitment.

Moser blamed ingrained British attitudes for the state of the country's educational system, but singled out the present government for an erosion of the social sciences in the United Kingdom. He argued strongly that the social disciplines are true sciences, denouncing the government's removal of the word 'science' from the title of the Economic and Social Research Council in 1983. Moser emphasized the importance of the social sciences' interaction with the 'harder' sciences in tackling problems such as global warming. **Peter Aldhous**

CARBON DIOXIDE SOURCE

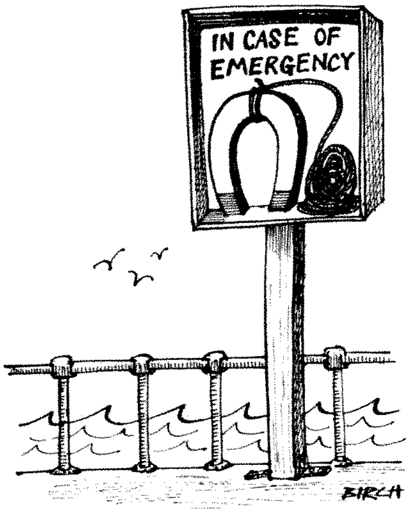
Filing plans for algae

London

PLANS to test the avant-garde idea, first mooted in the United States, that levels of atmospheric carbon dioxide could be reduced by sowing the oceans with tons of iron filings were unveiled by a marine biologist this week at the British Association for the Advancement of Science in Swansea.

Phillip Williamson of the Natural Environment Research Council's Plymouth Marine Laboratory announced plans for a "discreet" ocean test, which will be organized jointly by the Plymouth laboratory and the University of Rhode Island. The project would be smaller than the flagship proposal from a group of US researchers, which is currently under consideration by the US National Academy of Science, to deposit about a ton of iron over some 10,000 square kilometres of sea.

Williamson is one of a growing number of researchers who believe that in principle 'fertilizing' the sea with iron filings could boost the growth of carbon-dioxide absorbing marine algae sufficiently for significant amounts of the greenhouse gas to be sucked out of the atmosphere. Their belief stems from demonstrations of iron-induced algal growth in bottled sea water.



But the iron-filing idea has met with opposition from environmental groups such as Greenpeace who view it as a "bizarre technical fix" with unforeseen and potentially dangerous side effects. Its protagonists say that it is essential to find out now, whether iron filings could be used to bring down carbon dioxide levels in the event of a global warming emergency. And the only way to do that, they argue, is by conducting a small-scale test in the sea. **David Concar**

BIOTECHNOLOGY

Cetus forced into staff cuts

San Francisco

IN what appears to portend a change in corporate strategy, Cetus, the California-based biotechnology company, last week announced the resignation of president and chief executive officer Robert A. Fildes and the lay-off of 100 of its 950 workers. The news comes just three weeks after the refusal by a US Food and Drug Administration (FDA) advisory panel to recommend for approval Cetus's prize cancer drug, interleukin-2 (IL-2) (see *Nature* 346, 501; 1990).

The company attributed Fildes's resignation on 16 August to "differing views regarding the company's priorities", which biotechnology analysts take to mean that company officials were unwilling to continue Fildes's strategy of risking much of the firm's future on the much-delayed FDA approval of IL-2. Ronald E. Cape, who has replaced Fildes as chief executive officer, indicated that Cetus will pursue "alliances" with other companies in order to build business and reduce its risks. A spokeswoman denied that Cetus is considering selling off any parts of the company.

Announcement of how the workforce cuts will be made is expected by mid-September. Meanwhile, company officials are still trying to schedule their next meeting with the FDA to determine what additional data on IL-2 will be required, or whether additional clinical trials are necessary, which would lead to an even longer delay. Analysts now estimate that approval of the drug will be delayed for 12 to 18 months. Elizabeth Schaefer

CLEAN TECHNOLOGY

Councils to cooperate

Swansea

THE UK research councils have set up a unit to provide a focus for developing new products and processes that do not harm the environment.

The Clean Technology Unit, established by the Science and Engineering Research Council and the Agriculture and Food Research Council, will initially provide £1 million to top up 40 existing projects relevant to clean technology.

Announcing the formation of the unit at the British Association's conference in Swansea on Monday, its head Dr Nicholas Lawrence said this would rise to £10 million per year.

The first specific research projects will be announced in early 1991, and will focus on three areas: new processes that are inherently cleaner, modifications of existing processes to reduce or eliminate pollution and retrofits to deal with past pollution problems such as landfill sites.

Nuala Moran

A genuine global partnership?

S. S. Yamamoto

Japan is considering a huge financial investment in the US Superconducting Super Collider. But other types of contribution, to this and any international project, are equally important.

A HIGH-LEVEL delegation from the US Department of Energy recently visited Japan with a letter from President Bush to Prime Minister Kaifu soliciting about $1.6 thousand million for the construction of the Superconducting Super Collider. The request was made in the spirit of the agreement between Bush and Kaifu to form a 'global partnership'. But I have serious doubts as to whether financial support of the Superconducting Super Collider falls into the category of global partnership. Although the United States invite foreign participation where money and manpower are concerned, to the best of my knowledge they do not invite participation on an equal basis in scientific, technical and administrative matters. By definition, a global partnership must involve such participation. If the past record of the US–Japan cooperative programme in high-energy physics is anything to go by, involvement of Japanese scientists will be secondary to the country's financial contribution in the case of the new project.

The US government is not to blame. The problem is Japan's. An impediment to genuine international collaboration is the reluctance on the part of the Japanese government to provide enough money for its physicists who work on collaborative research projects overseas, and the reluctance on the part of Japanese universities and research institutes to let their people, particularly senior people, spend an extended period overseas to participate fully in an experiment. Junior physicists and graduate students, some of whom are on the payroll of US institutions participating in the experiment, must bear the burden of carrying out the work without much guidance or leadership. This is detrimental not only to the education and training but also to the significance of the scientific contribution of Japanese teams.

Wrong priorities

I vividly remember the comments of Professor Robert Wilson, a former director of Fermilab, in his talk on international cooperation in high-energy physics at the 1986 meeting of the Japanese Physical Society in Tokyo. After lauding Japan for its active participation in collaborative experiments in the United States, but without stating whether Japanese physicists had made any significant scientific contributions, he said, "and I particularly welcome your money". This candid statement succinctly reveals the state of many of the US–Japan collaborative experiments then in progress. The situation is not very different now.

Ninety-one Japanese physicists have agreed to participate in the Superconducting Super Collider project, but some of these are from small groups of one or two and not likely to participate in a significant way. I see no prospect for improvement. There are only a few postdoctoral fellowships provided by the Japan Society for the Promotion of Science to send young PhDs to work on international collaborative projects for a certain fixed period without tenured appointments. Therefore, it is likely that only a small fraction of the 91 physicists will be able to work on the project full-time. Under these circumstances, I feel the expenditure of nearly $2 thousand million is actually detrimental to the sound development of high-energy physics as well as other scientific disciplines in Japan for the following reasons.

Japanese physicists will be paying an entrance fee to join the project without really being able to make significant scientific contribution. Under the guise of US–Japan collaboration, some Japanese high-energy physicists have deprived themselves of an opportunity to do their own experiments, and some graduate students have been deprived of opportunities to work closely with their supervisors. In addition, if a sizeable fraction of the rather few university-based high-energy physicists are engaged full-time in the Superconducting Super Collider, how will their graduate students obtain PhDs? How can the domestic high-energy physics programme continue adequately if $1.6 thousand million is spent on the Superconducting Super Collider? To educate and train future physicists, the number of university-based high-energy physicists must be increased, and Japanese high-energy accelerators must remain operational and new machines built. Some Japanese physicists believe that in return for the contribution to the Superconducting Super Collider, all future high-energy accelerator projects in Japan, such as the Japan Linear Collider, should be abandoned, and high-energy physics experiments should be done abroad. Such an arrangement will have untold ill consequences for Japanese high-energy physics.

There is also the question of allocating funds for collaborative research projects in other countries, notably for LEP at CERN. There is already a successful collaboration in a multinational project called OPAL at LEP involving physicists from the University of Tokyo. At present, seven of these are working at CERN for extended periods. Some have been there for many years, and the cost of supporting them is borne by the university from a specially allocated budget. The capital expenditure for this collaboration is limited to the detector cost, but the case of the Superconducting Super Collider may cause CERN to request contributions from Japan to its operating funds. To have more balanced global collaborations, I think some of the money for the Superconducting Super Collider should be spent on this and other future international collaborations.

Political aspects

Finally, because the United States asked for money before adequate discussion among Japanese physicists as to the desirability and extent of their participation, the Superconducting Super Collider case has begun to take on a political aspect. A project of this magnitude is bound to be political to some extent, and of course physicists alone cannot decide the extent of the Japanese contribution. But if political considerations overwhelm the scientific ones, the project will bode ill for future international collaboration.

So should Japan contribute to the Superconducting Super Collider? My answer is a qualified yes — qualified because if the Japanese contribution is to be mostly monetary, my answer is no. But as the project is deemed to have significant scientific merit worthy of an enormous investment by many distinguished physicists around the world, and because there is strong interest in it on the part of some of the Japanese high-energy physics community, my answer has to be yes. The US government and high-energy physics community should insist that the Japanese contribution is scientific as well as financial. Because large-scale international collaborations are inevitable in many fields of basic and applied research, it is imperative that this case should be carefully discussed by physicists and government officials of both countries. □

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Problems with telescopes

SIR—I should like to comment on the problems with the Hubble Space Telescope (HST).

The primary mirrors of large modern telescopes are tested in autocollimation at their centres of focus, the error being determined by interferometric or Hartmann type analysis. Because primaries of short focal length are strongly aspheric (slightly hyperbolic for Ritchey-Chretien telescopes), the autocollimated image suffers from strong aberration, which is compensated during the test by a 'compensation' or 'null' system. This technique was invented by the English amateur Horace Dall in 1947[1].

The null system must be correct to very high precision, not only in its design and manufacture but also in its position in the test set-up, otherwise a systematic error arises which is spherical aberration in its classic form.

The testing of convex secondary mirrors, which cannot form real images, is a subject in its own right[2], but all test methods have tolerances which, if they are not respected, will lead to similar spherical aberration. But there is one test of secondaries (together with the whole system) which effectively ensures that matching error cannot occur: this is the Pentaprism test. The Pentaprism test is essentially a one-dimensional substitute for the autocollimation test against a plane mirror. The Pentaprism deflects small parallel beams about 90° and has the property that the deflection is unaffected by inevitable rotation errors of the Pentaprism as it is moved across a diameter of the pupil. The Pentaprism test will ensure only that the two mirrors will in combination produce an image free of spherical aberration at the nominal Cassegrain focus. Various other tests exist[2] for faults such as astigmatism and high spatial frequency errors ('ripple'). The Pentaprism test was probably first invented by Wetthauer and Brodun in 1920[3], and has been systematically used by a few manufacturers, for example REOSC of Paris[4] or Korhonen in the recently completed 2.5-m Nordic Optical Telescope. It was used in the United States in 1939 by Hendrix and Christie[5] for Schmidt systems, and was described by Hochgraf in 1969[6].

It seems surprising that this test, which is simple and cheap to set up (the telescope axis can be vertical or horizontal) was not applied to the HST; it would certainly have revealed the error.

But its use is by no means general in telescope-making, which explains why matching error, often very large, has been a common technical error. It affected the Canada–France–Hawaii Telescope, for which the spherical aberration was successfully corrected by bending the secondary.

The ESO New Technology Telescope (NTT) also had a matching error, which was provoked by a systematic error in the positioning of the compensation systems used to test the primary. Although this error was small (1.8 mm in a test distance of ~ 15.4 m) the wavefront error caused by the spherical aberration thus introduced was ~ 3,000 nm. This produced an image containing 100 per cent of the light in 0.71 arc seconds. Although this was outside the NTT's passive specification, we were able to correct it completely — to less than 0.1 arc sec, the general 'noise' level of the system — by the first (fixed) level of the active optics system[7].

The Pentaprism test had been proposed by ESO to the manufacturer, Carl Zeiss, but was dropped by mutual agreement because a double test system (visible and infrared) gave a cross-check of the individual mirror tests. Had a matching error existed outside the dynamic range of the NTT;s active optics system, it would have been revealed by this comparison. Further details will be given in the *ESO Messenger*, September 1990.

The HST, by contrast, has a very stiff, lightweight, egg-crate type of primary, and the dynamic range afforded by the 24 actuators is far too small to be able to correct an error in the primary of the magnitude found. Because the HST is effectively a passive telescope as far as correcting the primary is concerned, the Pentaprism test would have been the best guarantee against matching error. It is reliable, simple and cheap.

It has been said that the current performance of the HST is as good as the best ground-based telescopes. This is certainly not true. Apart from the NTT, which because of its active optics routinely produces images smaller than 0.5 arc second at the excellent site in La Silla, Chile, there are several passive telescopes, including the William Herschel Telescope in La Palma, capable of producing sub-arcsecond images under favourable seeing conditions. By contrast, the HST has an image size of at least 1 arcsecond from the spherical aberration alone.

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Foreign aid

SIR—The British Natural History Museum (NHM) can apparently no longer find the necessary £20 million or so annually to fund its present level of activities, much less pay for needed renewals and expansions. Yet it is an institution "of great international importance", its collections belonging "to the world, not just to London or the British Isles" (Adam Urbanek and Michael Novack; *Nature* **345**, 656; 1990). This suggests a solution to the present crisis. If the United Kingdom is now too poor to finance the NHM adequately, let us rename it the International Museum of Natural History, and arrange to support it with funds from the International Union of Biological Sciences, the Smithsonian Institution and any other international or national organizations and individuals who recognize the necessity for such action at the present time.

Meanwhile, Britain feels that it *can* afford Trident missiles, a sad indication of where its priorities lie today.

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Correction

In the opening paragraph of his letter about the Natural History Museum, Des Griffin (*Nature* **346**, 99; 1990) was referring to commentary on the subject in general rather than to Sir Walter Bodmer's Commentary in *Nature*. ☐

Need for religion

SIR—The effort expended by scientists to prove God to be a myth and religion to be a superstition would be more fruitfully spent in finding out why belief in transcendental power is so widespread: just about every culture includes a religion. I can think of two reasons:

(1) Religion helps societies to function more effectively, giving them a competitive edge over atheists.

(2) Some humans are dimly aware of another dimension of this Universe, or even of some other continuum, that they call God. This awareness helps them make more constructive decisions than those of people lacking such a faculty.

A simplistic view indicates that, in the struggle for survival, atheists should have prevailed, as religious practices are a drain on resources. Yet it is the atheistic societies that failed to survive. Religion must have something going for it. Instead of pretending that it does not exist, we should try to see what makes it tick.

ANDREJS BAIDINS

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Assertive sentence titles

SIR—If it is such a bad thing for molecular biologists to use declarative sentences in journal titles as Rosner asserts[1], why have they made so much progress, at a time (1982–89) when this deplorable process so increased in popularity that only *Nature* seems to have escaped from the floodtide of assertive sentence titles (ASTs)?

Is it the result of a rise in confidence in a process which is so successful that buyouts and allegations of insider dealings in biotech companies are as much news in *Nature* as scientific budgets[2]?

Or has success so emboldened biologists to test their work by the methodology of proof by falsification that they have confidence to use declarative English? And are not declarative sentences much easier to prove erroneous than open-ended questions, which really are akin to playing tennis with the net down — no rules need apply? An example will suffice.

Rosner erroneously asserts that ASTs are often patently unprovable, for example declarations which cite that a process requires a certain component; Rosner claims that only when all possible conditions are tried can they be substantiated. But in fact, only the negation of the condition need be demonstrated. Declarations that a process requires or does not require a certain component are substantiated through the conspicuous use of control experiments.

The withdrawal by Fleischmann and Pons of their cold fusion paper submitted to *Nature*[3] had less to do with the absence of title citations in this journal's references, as one could infer from Rosner's hypothesis, than the referees' criticism of the need to control for possible neutron contamination from other sources, such as cosmic rays[4]. The cold fusion account from the Brigham Young group was published[5] after it corrected for possible background contamination in the results.

ASTs make for progress only when the underlying papers survive the same process of scrutiny used to test scientific hypotheses in other disciplines. It is the open-ended nature of the falsifiability criterion and not grammatical constructs that make for the tentativeness of scientific assertion and keep the scientific endeavour a process and not a product.

EDWARD J. KROWITZ

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1. Rosner, J.L. *Nature* **345**, 108 (1990).
2. Gershon, D., *Nature* **345**, 102 (1990).
3. *Nature* **338**, 691 (1989).
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5. Jones, S.E. *et al. Nature* **338**, 737–740 (1989).

Relativity values

SIR—Stefan Marinov's apparatus designed to test relativity (*Nature* **346**, 103; 1990) is clearly similar in principle to the electromagnetic flow-meter, which works by measuring the voltage induced by fluid moving in a magnetic field.

Assuming that such an instrument is set up to give zero output for zero flow, a reversal of flow direction reverses the polarity of the output. I would therefore say that "the polarity of the slider depends on the direction of the current in the concentric circuit, *and also* on whether the slider moves clockwise or anticlockwise".

We use electromagnetic flow-meters both as current-meters, in which case the instrument is fixed and the water moves, and as ships' logs, where the water may be stationary and the instrument moves. However, I have not yet managed to get an output from such an instrument by putting it in a bucket of water and marching across the laboratory with it.

A. J. BUNTING

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Metric false trail

SIR—The influence of the metric system both in science and in everyday life has also been responsible for a fatal flaw in conventional computation. This is that the binary code of digital computers is exponentiated in radix ten numbers, instead of base two numbers. The result is that every switching operation carried out at the system clock frequency of a computer is essentially "bringing down the exponent" in order to carry out conversions between the respective number bases. All information is expressible in bits (binary digits) and there is therefore no reason why computation cannot be carried out directly in the binary system. The ineluctable advance of the metric system supposed by your correspondent C. H. Evans (*Nature* **345**, 658; 1990) will come to a permanent halt as soon as it is realized that the Emperor at the head of the procession has no clothes.

B. E. P. CLEMENT

Everest Drive,
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Powys, NP8 1DH, UK

Never again

SIR—I should like to comment on the review by Robert N. Proctor (*Nature* **344**, 502; 1990) of Paul Weindling's book *Race and German Politics between National Unification and Nazism 1870–1945*).

J. F. Lehmanns Medizinische Buchhandlung GmbH was founded in 1981 as an independent company; in the same year, the five Rothacker bookshops were bought by the Deutsche Arzte Verlag. The name J. F. Lehmanns was chosen for these bookshops without anyone being aware of the infamous past of this name.

For many years after the Second World War, high-level and specialist medical books with a high reputation in scientific circles were published under the imprint J. F. Lehmanns. This publisher has since been amalgamated into the well-known company Springer Verlag.

It was certainly a mistake not to research the background of this name. The past nine years have, however, proved that our company and its employees cannot be associated with any thought of nazism, however expressed. As far as it is in our power to determine, fascism and other extreme ideologies will never again stand a chance with us.

MANFRED HAHN

J. F. Lehmanns,
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D-8400 Regensburg 1, FRG

BSE gene-linked?

SIR—Managed cattle breeding, progeny testing and sire selection are commonplaces of British livestock husbandry. As a result, many of our herds have considerable common ancestry; and with widespread and cheap artificial insemination, it is not impossible for one bull to impregnate tens of thousands of cows, many of them in successive generations.

Bovine spongiform encephalopathy (BSE) is a peculiar disorder. It appeared abruptly, nationally and with no obvious geographical foci. If it is caused by an infectious agent, then this is both difficult to detect and difficult reliably to transmit. Indeed, those animals to which it is irregularly transmissible appear to come from the epidemiologically 'at risk' group. We see it as a transmissible disease primarily by analogy with scrapie and other neuropathologies; and we have sought an explanation for the rapid onset of diagnosis in the use of offal in stock feeds. One could, however, also see BSE as a genetic disorder brought to prominence by the extensive inbreeding and commonality of germ lines.

In view of this, it would be interesting if a researcher with access to the appropriate databases were to run a check on the ancestry of those cows diagnosed as having BSE. If BSE were to be shown to be the outcome of inbreeding rather than the transmission of an infectious agent, the economic implications — and the policy measures that should be followed — would have implications on a national scale.

O. H. G. SPARROW

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London SW6 3TF, UK

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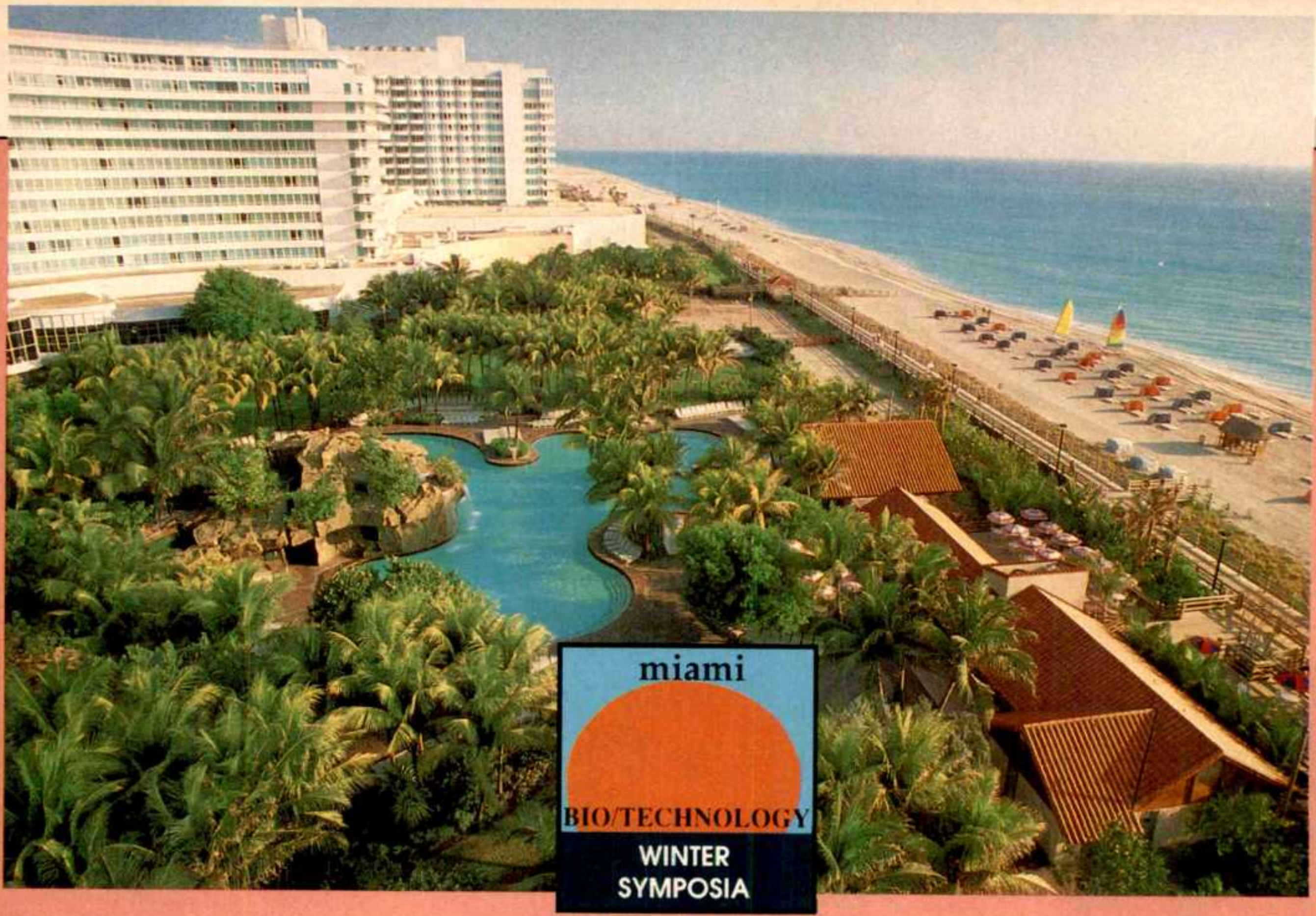

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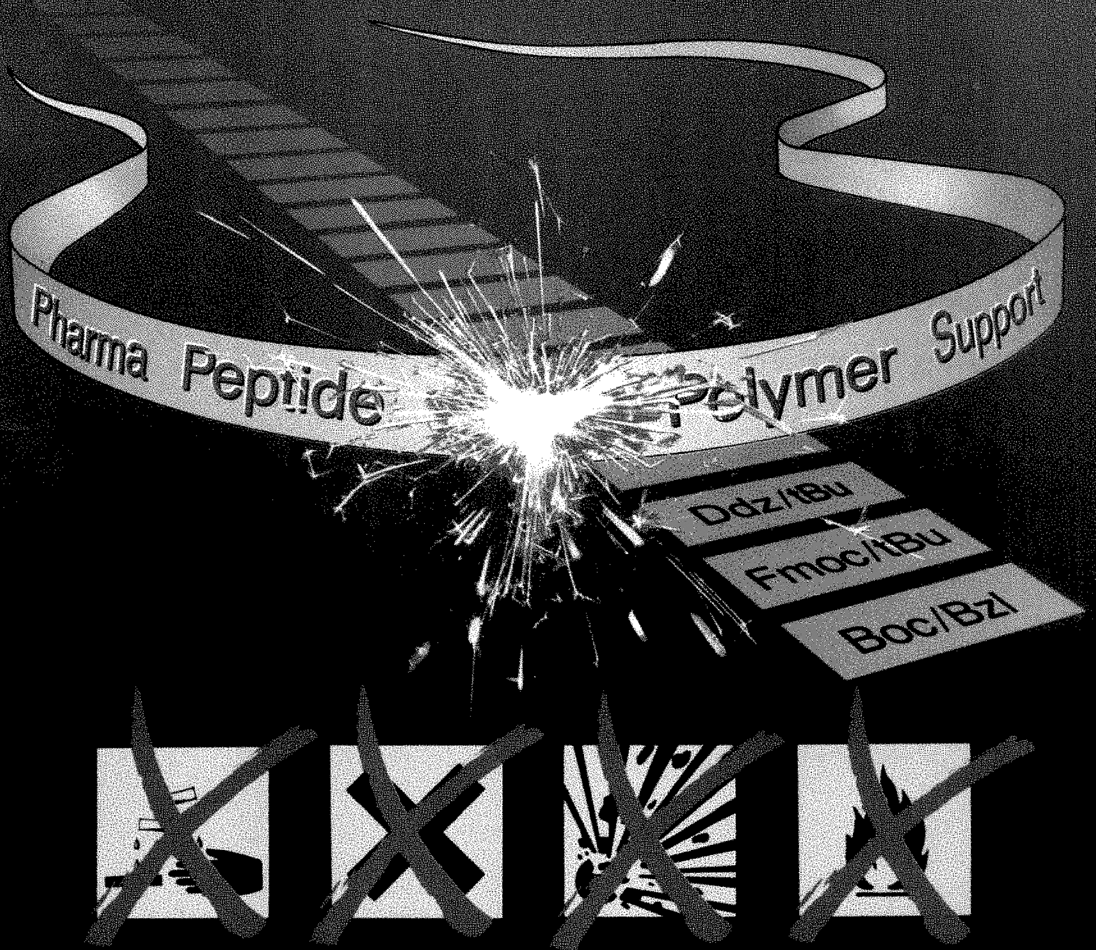

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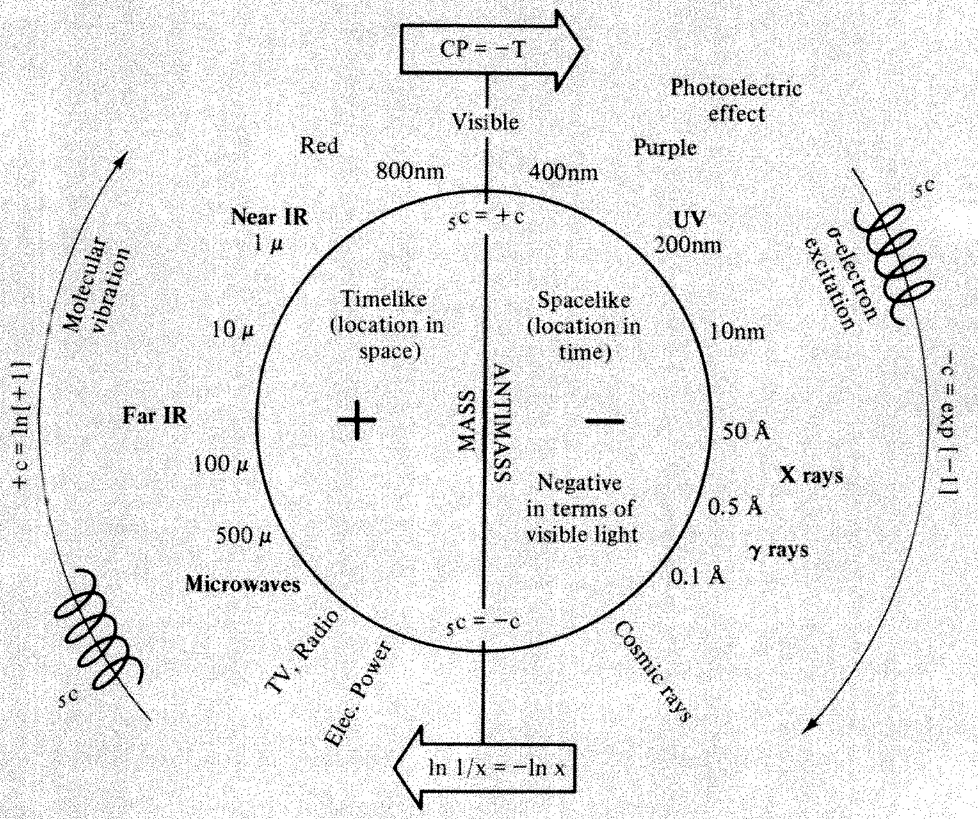
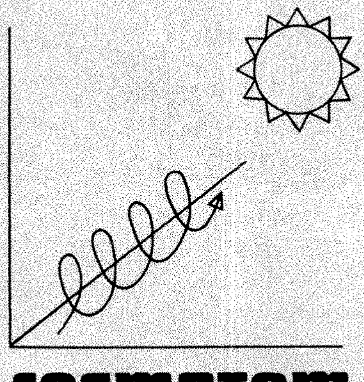


Cf. Blackett & Occhialini (1933)
Cosmic rays → e^+e^- etc. = p^+p^- in 5D.

IAN McCRIMMON



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Towards a history of the Sun?

There seems to be just a chance that geochemists could reconstruct a record of the Sun's activity as a main-sequence star from measurements of the abundance of xenon-126 in rocks.

HERE is a conundrum for climate modellers and for the global warming community in particular: if it is true, as astrophysicists say, that the luminosity of the Sun has increased by 40 per cent in the past 5,000 million years, how is it possible to explain that the Earth has only just (since 18,000 years ago) emerged from a major glaciation? The question is not new; it has been on people's minds for decades. The need to answer it has been made compelling because the climate models that predict global warming have not yet been turned systematically and successfully onto historical data, with the consequence that the sceptics of global warming can fairly jeer: "If you cannot explain why there was, say, a Little Ice Age in Europe in the eighteenth century, why should we take seriously your predictions of the future?"

The postdiction problem is not, of course, as simple as it sounds. First, there is the question of what data to use. Ice-core measurements may have helped to define the composition of the atmosphere during relatively recent climate fluctuations, but the data are necessarily less precise than from measurements of the modern atmosphere. Estimating the composition of the Archaean atmosphere is, by extension, hardly distinguishable from guesswork.

The input of energy from the Sun is another imponderable. Variations of the Earth's orbital elements can be calculated well enough, at least over intervals of a few million years, to be included in climate models. But while satellite measurements of the solar constant suggest that fluctuations of the output of energy from the Sun are too small to account even for the barely distinguishable climatic trends in recent decades, it is always possible that earlier (larger) climatic fluctuations were somehow linked with variation of solar output — the coincidence of low winter temperatures in eighteenth century Europe and the Maunder sunspot minimum often seems too good to be true.

Over much longer intervals, there seems no doubt that the solar constant has changed, and indeed increased substantially. The Sun has been a main-sequence star for at least 4,500 million years, during which time hydrogen has been converted into helium in the core of the Sun, thus increasing the opacity of the material of the core, the central temperature and, eventually, the luminosity. Astrophysicists will say that the structure and, indeed, the evolution of the Sun is one of the fixed certainties in their otherwise uncertain world. That, of course, is why there has been such consternation in the past 20 years because the number of neutrinos reaching the surface of the Earth is smaller (by between a half and two-thirds, according to energy) than the calculations suggest. Another difficulty is that, if there has been a 40 per cent increase of the solar constant in 5,000 million years, why have we only just emerged from an ice age — a period much colder than at the end of the Cretaceous, for example?

Despite astrophysicists' confidence in their picture of the evolution of the Sun, there are of course ways in which they might have been misled. One of the underlying assumptions in the astrophysical model of the Sun, for example, is that the hydrogen-burning core is thermally stable, or that convection is not an important cause of mixing. If the assumption is incorrect, there could well have been uncounted variations of solar constant in the past 5,000 million years.

Whence the ambition of W. C. Haxton, from the University of Washington at Seattle, to find a way of estimating the time-course of solar luminosity from measurements on the surface of the Earth (*Phys. Rev. Lett.* **65**, 809; 1990). How could that be possible? Only if the terrestrial consequences of the solar neutrino flux could be significant. That drives Haxton to geochemistry. His ingenious proposal is for a scheme for the measurement of the past neutrino flux from the Sun by means of the conversion of tellurium into xenon isotopes under the influence of energetic solar neutrinos.

That solar neutrinos should have had terrestrial consequences should be no surprise. The known deficiency of solar neutrinos was, after all, first recognized by the conversion of chlorine into neon isotopes by solar neutrinos. Since then, much ingenuity has been spent on other schemes for using terrestrial isotope abundances as measures of the neutrino flux, both as detectors for measuring the contemporary flux and as geochemical proxies for the past abundance of neutrinos. Haxton points out that there is already a geochemical scheme for the measurement of past neutrino fluxes based on the isotopes of technetium (element number 43, which has no stable isotopes). Indeed, ^{98}Tc is produced exclusively by neutrino interactions, so that its concentration at any time could be a measure of neutrino flux over periods of time comparable with the half-life of the isotope. The obvious snag is that the technetium isotopes are so rare as to be hardly measurable and are, in any case, unstable.

Haxton's scheme is based on a search through the table of isotopes for a material likely to be made almost exclusively by neutrino interaction and which, although itself unstable, will yield a stable daughter product. His suggestion is that the conversion by neutrino interaction of tellurium-126 (^{126}Te) into xenon-126 (^{126}Xe) may be just enough to provide a yardstick of past solar activity. Briefly, sufficiently energetic neutrinos will convert ^{126}Te nuclei into ^{126}I nuclei, a proportion of which then decay into stable ^{126}Xe, the rarest of the xenon isotopes (with a natural abundance of 0.089 per cent).

The outcome is a proposal for searching for excess abundance of ^{126}Xe in terrestrial rocks containing tellurium, gold ores for example, and using that to infer the past flux of solar neutrinos with energy above the reaction threshold (which would exclude all solar sources of neutrinos except the most energetic, those derived from the decay of ^{8}B). In principle, it should be possible to use the ^{126}Xe concentration of tellurides as a measure of the total solar neutrino flux since the formation of the rocks concerned, or the time at which they were last molten. The comparison of results from rocks with different ages could then, in principle, yield an estimate of the time-course of the neutrino flux.

Haxton is the first to acknowledge that the task will not be easy. An assay based on the measured isotope excess, estimated at 0.1 per cent, of the rarest isotope of a rare gas is hardly likely to be everybody's idea of a simple project. Indeed, although Haxton points out that ^{126}Xe is shielded from contamination by cosmic-ray neutron conversion of nearby isotopes by their rarity (and instability), the background problem is bound to be horrendous. But, luckily, geochemistry is sustained by the zealous enthusiasm of a handful of people, typified by Edward Anders at Chicago, who regard problems such as these as interesting challenges. It will be great fun if Haxton's ingenious scheme turns out to be a usable guide to the history of the Sun's evolution.

John Maddox

ONCOPROTEINS

GAPs in understanding Ras

Michael H. Wigler

RAS proteins play a central role in neoplasia and growth control, yet we do not understand how they are controlled or what they control. The papers by Downward *et al.*[1] and Zhang and colleagues[2] on pages 719 and 754 of this issue indicate that the enigma of Ras may be yielding.

Mammalian *ras* genes were first discovered as the oncogenes of acutely transforming retroviruses[3]. Subsequently, *ras* genes with activating mutations were found in many human and rodent tumours, providing the first solid evidence that a common biochemical defect might underlie cell proliferation, or neoplasia[4]. The importance of *ras* in the scheme of growth control and neoplasia is further underscored by observations that *ras* function is required for many other oncoproteins, such as tyrosine kinases, and growth stimulants, such as the tumour-promoting phorbol esters, to have effect[5]. Moreover, *ras* genes are evolutionarily the most highly conserved oncogenes yet found, and occur in all eukaryotic organisms where they have been sought. There are great similarities between the function and regulation of *ras* in microorganisms and vertebrates, but this oncogene's main function in the yeast *Saccharomyces cerevisiae*, which is the stimulation of adenylyl cyclase, does not apparently parallel its function in vertebrates[6,7].

The *ras* genes encode a family of small, closely related proteins found at the inner surface of the plasma membrane where they can bind guanine nucleotides and slowly hydrolyse GTP to GDP[4]. Only when they are bound to GTP can the proteins fulfil their role in cell proliferation[8,9]. Rates of GTP hydrolysis by wild-type Ras protein, but not oncogenic protein, are vastly elevated *in vivo* relative to rates measured for purified Ras proteins *in vitro*[9]. These discrepancies led to the discovery of GAP, a GTPase-activating protein found ubiquitously in cells. GAP is a large protein with relative molecular mass 110,000 (110K). The carboxy-terminal 40K acts catalytically upon wild-type Ras proteins to stimulate their hydrolysis of bound GTP, but fails to stimulate GTP hydrolysis by mutant oncogenic Ras[10].

Regulation

GAP is thus an excellent candidate to be an upstream regulator of Ras protein, capable of downregulating Ras, thereby keeping it inactivated in the GDP-bound state. Oncogenic Ras proteins would escape this regulation. The reports in this issue[1,2] strongly support this notion. First, Zhang *et al.*[2] report that transfection of the GAP gene inhibits morphological transformation in NIH 3T3 indicator cells by the normal H-*ras* gene, but not by the activated mutant v-*ras* gene. These results provide the first direct evidence that GAP can inhibit Ras function in mammalian cells. It was already known that GAP has sequence similarities to the *IRA* gene products of *S. cerevisiae*, which downregulate yeast *ras* genes[11]. Mammalian GAP, if expressed in yeast, can complement the loss of the *IRA-1* gene, and can inhibit the wild-type mammalian H-*ras* but not the activated oncogenic form when coexpressed in yeast[12].

Downward *et al.*[1] provide a deeper insight into the factors that regulate Ras function. They report for the first time a physiologically relevant event that leads to activation of Ras: stimulation of the immune system's T-cell receptor. The amount of Ras in the GTP-bound state in T cells rapidly rises tenfold when cells are stimulated with PHA or a monoclonal antibody directed to the T-cell receptor. Stimulation of the T-cell receptor is thought to activate phospholipase C, which can generate at least two intracellular messengers: diacylglycerol, which activates protein kinase C, and inositol phosphates, which can mobilize intracellular calcium. Downward *et al.* demonstrate that the activation of protein kinase C by the tumour-promoting phorbol esters increases even more rapidly the proportion of Ras bound to GTP in T cells.

Similar but lesser effects were observed in other cell types. In principle, an increase in the ratio of Ras bound with GTP to Ras bound with GDP could result either from an increase in the rate of nucleotide exchange on Ras or from an inhibition of GTP hydrolysis, owing, for example, to an inhibition of GAP activity. In fact, Downward *et al.* observed no alteration in the rate of nucleotide exchange on Ras upon treatment with phorbol ester, but they found a rapid decrease in GAP activity in treated cells, suggesting that a GAP-like protein, lying in the signal transduction pathways of T-cell stimulation and protein kinase C activation, regulates Ras.

The question of the placement of GAP in the Ras signal transduction pathway is not so easily resolved. Mutant forms of Ras which fail to have effector function also fail to interact properly with GAP, leading to the hypothesis that GAP may indeed be a target of Ras action[13,14]. This remained an intriguing but weak hypothesis until recently, when Yatani *et al.*[15] reported that GAP and Ras seem to cooperate in the inhibition of coupling between muscarinic receptors and atrial potassium channels. Each protein, purified, can inhibit this coupling alone, as can be demonstrated by an *in vitro* patch-clamp preparation, and antibodies to either component can block the activity of the other. Yatani *et al.* speculate that a Ras·GAP complex may alter membrane phospholipid metabolism.

Effector function

Do the results of Yatani *et al.* conflict with those of Zhang *et al.*? Not necessarily. The latter are compatible with two possibilities. First, endogenous levels of GAP in cells may not be limiting for Ras effector function. High levels of exogenous GAP might decrease Ras function if the amount of GAP-bound Ras exceeds the levels of a stoichiometric target for GAP. Second, Ras might have more than one effector function in cells. It is clear from the comparative studies that Ras has had at least two effector functions in the course of evolution. Moreover, evidence has accumulated that Ras has more than one primary effector function in *S. cerevisiae*[16]. These observations serve as a warning that Ras effector functions in mammalian cells are likely to be complex.

Hope for the resolution of the function of GAP has flared anew with the riveting report by Xu *et al.*[17] that *NF-1*, the locus involved in hereditary neurofibromatosis, encodes a protein that is similar to GAP and to the yeast *IRA-1* gene product[18] (see also Ponder's News and Views article on page 703 of this issue). The similarity between the *NF-1* gene product and GAP is restricted to the domain that catalyses accelerated GTP hydrolysis by Ras. But, the similarity between NF-1 and IRA-1 extends well beyond this region, and NF-1 is consequently closer to IRA-1 than it is to mammalian GAP. This result suggests that there may be a family of GAP-like molecules which may interact with Ras.

Patients with a single copy of the NF-1 disease locus are prone to sporadic benign growths of neuroectodermal origin, and hence, if hereditary neurofibromatosis

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follows the pattern of hereditary retinoblastoma, the NF-1 gene may be an anti-oncogene. If its product holds in check the activity of Ras, or a Ras-like protein, the sporadic loss of the good allele in the cells of NF-1 patients would then lead to aberrations in the growth control of these cells due to the constitutive activation of Ras. In this interpretation, the *NF-1* gene product acts as an upstream negative regulator of Ras.

There is, however, a contradictory interpretation. In some cells of neuroectodermal origin, Ras stimulates differentiation and blocks proliferation[19,20]. If *NF-1* encodes the target for Ras action in neuroectodermal cells, the loss of this product might also lead to uncontrolled cellular proliferation. Thus, for now, the striking news about *NF-1* is perfectly ambiguous. ☐

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SILICON CLUSTERS

All surface and no activity

Tony Stace

ELEMENTARY considerations of solid geometry show that as the radius of a sphere is reduced, the ratio of surface area to volume increases. If this picture is extrapolated to an atomic scale it is easy to appreciate that for a small collection of atoms — a cluster — such as Si_{24}, the majority of atoms reside on the surface. Given such circumstances it is, therefore, surprising to note that in a recent study of silicon clusters, Jarrold *et al.*[1] observed the clusters to be far less reactive for molecular oxygen than the surface of bulk silicon.

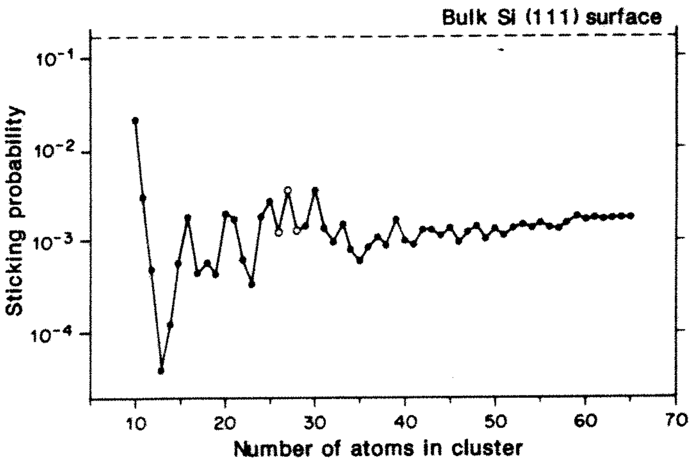

FIG. 1 Estimated sticking probability for the collision between an Si_n^+ cluster and an oxygen molecule as a function of cluster size. Shown as a dashed line is the result for the bulk Si(111) surface. (From ref. 1.)

The technical importance of silicon in the fabrication of electronic devices has meant that processes like the oxidation of well characterized surfaces have been thoroughly investigated. With the trend towards microelectronics and the possibility of constructing nanometre-scale (10^{-9} m) devices[2], there is clearly a need to identify how the chemical and physical properties of a bulk metal or semiconductor change when reduced to atomic proportions; the study of clusters could fulfil that requirement.

Clusters from refractory materials, such as silicon, are best prepared using laser vaporization; an intense pulse of laser radiation strikes a metal surface, vaporizing material which is then caught in the flow of a buffer gas such as helium. The gas cools the vapour, and promotes nucleation, resulting in a selection of clusters of various sizes. As will be seen, the efficiency of the cooling process could have a key role in determining the reactivity of the clusters.

A complicating factor in studying reactivity is that the comparisons are made between ionic clusters and neutral surfaces of bulk silicon. Experimental convenience dictates that ionic clusters are used; the electric charge means that clusters can be mass-selected and guided to the reaction zone. In the event, delocalization of the charge diminishes the magnitude of any electrostatic interactions between a cluster and a reactant molecule, and calculations suggest that there is very little difference between the structures of large neutral and ionic silicon clusters.

Similar experimental considerations apply to the reaction products: charged products are readily identified using mass spectrometry, whereas the nature of neutral fragments can often be inferred from the thermochemistry. Thus, in the case of a reaction between a silicon cluster ion and an oxygen molecule, the dominant steps are[1]:

$$Si_n^+ + O_2 \rightarrow Si_{n-2}^+ + 2SiO \text{ or } Si_2O_2 \quad (\text{for } n < 30) \qquad (1)$$

$$\rightarrow Si_nO_2^+ \quad (\text{for } n > 30) \qquad (2)$$

The absence of detailed information on any neutral reaction products, together with the observation of step (2), supports the approach taken by Jarrold *et al.*[1] in interpreting the experimental data in terms of sticking probabilities. On this basis, a direct comparison with bulk silicon[3] suggests that the probability of O_2 sticking on a cluster is lower by a factor of 100. Although the reactivity of smaller clusters with oxygen varies significantly with size (Fig. 1), beyond Si_{25}^+ the sticking probability is almost constant as a function of size.

Jarrold[4] recently compiled a list of other molecules, such as methanol and ammonia, whose reactivity varies markedly with the size of the target silicon clusters. Possibly the most dramatic examples have come from the work of Smalley and co-workers[5], who found that the reactivities of the clusters Si_{39}^+ and Si_{45}^+ with ammonia and ethene are several orders of magnitude lower than those of clusters one atom smaller or larger.

In a novel extension to those experiments, Smalley's group has shown[6] that the reactivity of a silicon cluster ion can be modified through laser-induced annealing. In particular, annealing renders Si_{39}^+ and Si_{45}^+ virtually inert with respect to ethene and ammonia. But note that Jarrold *et al.*[1] did not see a similar discrimination on the part of these large clusters against oxygen. Such marked differences in behaviour between what are in essence identical experiments suggests that it may be necessary to consider in detail the way the silicon clusters are produced.

Laser vaporization leads to the generation of a plasma consisting of atoms, ions and electrons. The gas pulse which entrains the vaporized plume quenches the plasma and initiates nucleation. But because the binding energy between silicon atoms is quite high (the heat of atomization is about 4.6 electron volts), it is possible to produce 'stable' clusters with quite high internal temperatures, and only through further collisions and/or evaporation will they become annealed. Depending on the exact conditions, three types of silicon cluster could emerge from the vaporization process: hot and possibly liquid-like; annealed, but to a metastable structure; or annealed to the ground-state structure.

Clusters from the first option might behave in a manner analogous to a hot surface, having low sticking probabilities owing to the rapid desorption of molecules. Beck and Andrews[7] recently provided evidence of a laser-induced phase transition between solid-like and liquid-like forms of silicon clusters. Bearing in mind that the melting point of a cluster could be at least a factor of two lower than that of the bulk material[8], estimates of the (photon) energy required to 'melt' a cluster may need to be re-evaluated. The possibility of the annealed options suggests the existence of structural isomers. These

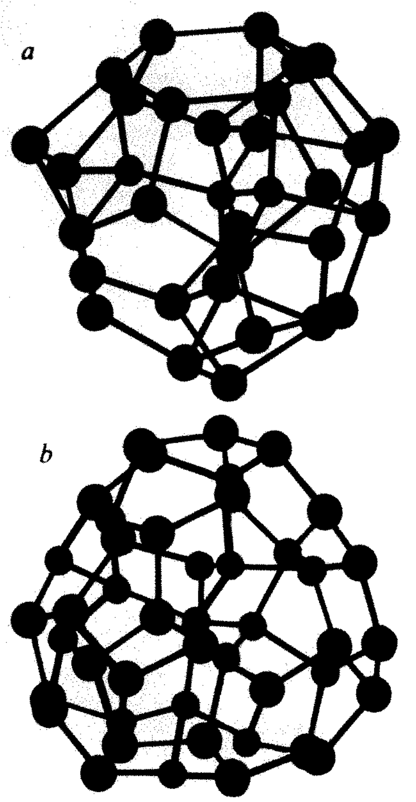

FIG. 2 Perspective views of Si_{33} (*a*) and Si_{45} (*b*). (From ref. 10.)

by several groups (see, for example, ref. 9) with reference to changes in the reactivity of niobium clusters.

It is, therefore, apparent that experimental conditions could strongly influence the type of reactive behaviour observed with metal and semiconductor clusters. Given that sufficient annealing does produce 'crystals' of silicon, it is clear from the results of Smalley and co-workers[5,6] that certain atomic combinations are very much less reactive than their immediate neighbours.

To account for such behaviour, Kaxiras[10] has suggested that certain combinations of silicon atoms, such as Si_{33} and Si_{45} adopt stable 'magic-number' structures (Fig. 2) which closely match the reconstructions observed for the silicon (111) surface in the bulk phase. The driving force behind surface reconstruction is the elimination of dangling bonds (unsaturated valencies), and it is a feature particularly characteristic of semiconductor surfaces.

But having identified stable structures, we appear to be left with a conundrum: if Si_{33} or Si_{45} have very high surface areas and the morphologies of those areas are thought to resemble the bulk silicon (111) surface, why is it that the clusters are orders of magnitude less reactive than the bulk? Could it be that clusters emerge from the vaporization–annealing process with internal temperatures that are higher than have previously been considered appropriate? ☐

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MEMORY

Maintenance is presynaptic

T. V. P. Bliss

LONG-term potentiation, a sustained enhancement of synaptic efficacy induced by high-frequency electrical stimulation of certain pathways in the neural mammalian brain, may well provide the neural basis for at least some aspects of memory. Two reports, one by Malinow and Tsien[1] and the other by Bekkers and Stevens on page 724 of this issue[2], present compelling new evidence that long-term potentiation is expressed as a persistent increase in neurotransmitter release. This result may clarify the long-standing debate about which side of the synapse is responsible for the maintenance of long-term potentiation, and will direct attention to the cellular machinery by which long-term changes in neurotransmitter release can be achieved.

The trigger for long-term potentiation (LTP) is almost certainly the postsynaptic entry of calcium through the voltage-dependent channels controlled by the *N*-methyl-D-aspartate (NMDA) subtype of glutamate receptor[3], a mechanism that provides a satisfying molecular explanation for just those properties of LTP that enhance its appeal as a memory model, namely associativity and specificity. By contrast, there has never been agreement on whether the locus of LTP expression is pre- or postsynaptic. The fact that induction reflects postsynaptic events does not by itself entail a postsynaptic location for the mechanism ultimately responsible for the sustained increase in synaptic efficacy. The debate has continued for many years between those favouring a postsynaptic mechanism, involving say changes in receptor number or sensitivity[4], and those, myself included, who prefer a presynaptic mechanism expressed as a sustained increase in transmitter release[5], with a position on the fence being a tenable and indeed often prudent option.

The latter two positions were made considerably less comfortable by reports from two groups in 1988 that the two components of the synaptic response, mediated by glutamate receptors of the kainate/quisqualate and NMDA subtypes, are differentially affected in LTP, there being little if any potentiation of the NMDA component[6,7]. This observation seemed to favour a postsynaptic mechanism, because an increase in transmitter release would be expected to affect both components, and indeed does so in paired-pulse facilitation and in post-tetanic potentiation (a short-term effect which, unlike LTP, occurs in most synapses). Proponents of a presynaptic mechanism were forced to search for *ad hoc* explanations such as a persistent downregulation of NMDA receptor/channel function following the induction of LTP. Now Malinow and Tsien[1] and Bekkers and Stevens[2] swing the pendulum towards the presynaptic side. These authors have applied the technique of whole-cell recording with patch electrodes to a quantal analysis of LTP in the hippocampal slice, and conclude that LTP is mainly presynaptic in origin.

Quantal analysis has long been the preferred way of establishing whether changes in synaptic efficacy are presynaptic or postsynaptic. The method exploits the discrete and probabilistic nature of the exocytosis of transmitter from vesicular stores at synaptic release sites to derive estimates of the binomial parameters *N* (number of release sites), *P* (probability of a nerve impulse leading to release from a given release site) and *a* (the synaptic response produced by a single quantum, usually identified with the contents of a single vesicle). *N* and *P* are presynaptic parameters, whereas *a* is postsynaptic (assuming constant packing of transmitter in vesicles). Analysis of changes in *N*, *P* and *a* following the induction of LTP can in principle indicate the locus of change.

The problem is that, unlike the neuromuscular junction with its single synapse, neurons receive thousands of connections at varying distances from the recording site in the cell body. This, together with the poor signal-to-noise ratio obtained with the high-resistance electrodes required to penetrate small cortical cells, greatly complicates the analysis[8], and prevented general acceptance of an earlier quantal analysis[9] which reached similar conclusions about the locus of LTP.

How have the two Californian groups[1,2] overcome these difficulties? Their chief success was to record from cells using low-resistance patch electrodes that allow an order-of-magnitude increase in signal-to-noise ratio. They use two methods to reduce the number of activated synapses: minimal stimulation to activate at most a few afferent fibres; and impalement of a hippocampal presynaptic CA3 pyramidal cell projecting to an impaled postsynaptic CA1 cell to allow a single synapse to be studied (the success rate in such an endeavour is extremely low[10] and Bekkers and Stevens resorted to dissociated hippocampal cultures for this type of experiment). Malinow and Tsien validated their approach by showing that it gives the expected answers in situations in which the answer is known. When transmission was enhanced by increasing extracellular calcium, there was a predicted increase in 1/CV (ref. 11), where CV is the coefficient of variation of minimal synaptic responses, a presynaptic measure depending only on N and P. Conversely, when a glutamate receptor antagonist was applied, leading to a decrease in synaptic transmission by a presumed reduction in a, there was no change in this measure.

In the case of experiments on hippocampal slices, both groups[1,2] emphasize that their analysis is restricted to a composite measure of presynaptic function involving both N and P. Their results thus do not preclude a change in a, and hence some postsynaptic involvement. But in the experiments with cultured cells, Bekkers and Stevens found that the binomial distribution gave a better fit than the Poisson distribution, and showed that the change was in P rather than in N. This result is important because although a relatively implausible postsynaptic model[2] can be constructed to accommodate changes in N, it is difficult to see how this can be done for changes in P. Bekkers and Stevens can therefore make a slightly stronger claim for presynaptic suzerainity than Malinow and Tsien, although the fact that different statistical models apply in hippocampal slices and in cultured cells carries an implicit reminder that different mechanisms could apply in the two cases.

One surprising observation, however, applies to both preparations. Bekkers and Stevens report a substantial variance in the amplitude of individual quantal events (miniature excitatory synaptic currents), in contrast to the constancy of such events in other neurons where it has been examined[8] — it is this additional variance that presumably obscures the appearance of peaks, corresponding to multiples of the quantal amplitude, in the amplitude distributions illustrated by Malinow and Tsien. In another paper[12], Bekkers *et al.* conclude that variability in miniature quantal events probably results from variation in vesicle size, and hence in the amount of transmitter released in each quantal event. But estimates of the number of glutamate molecules in a synaptic vesicle (about 3,000; ref. 11) are an order of magnitude greater than the estimates of the number of kainate/quisqualate channels which need to open to provide the conductance change underlying a miniature quantal event. (In slices, the mean miniature quantal conductance is 0.8 nS (ref. 12). Assuming a kainate/quisqualate channel conductance of 10 pS, fewer than 100 channels open during a miniature quantal event.) Thus, even if cooperative binding of transmitter molecules is required for channel opening, a single vesicle probably contains enough transmitter to saturate subsynaptic receptors, so it is not clear how vesicle variability contributes to quantal variance.

Is this the end of the story? The history of the field suggests not. It remains to be seen whether quantal analysis of components mediated by receptor subtypes can reconcile the new results with the differential potentiation of NMDA and non-NMDA responses[4,5]. Meanwhile, another report[13] of quantal analysis of LTP in the Schaffer collateral pathway, which links CA3 cells to CA1 cells in the hippocampus, concludes that LTP is expressed as a change in quantal size without change in quantal content. Back on the fence, seats are filling up fast.

Finally, a presynaptic site for the expression of LTP suggests that a signal molecule is required to carry information back across the synapse from the postsynaptic site of induction. One such candidate, arachidonic acid, has been identified[14], and it is certain that the two new papers will stimulate the search for others. ☐

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QUANTUM MECHANICS

A quantum time machine

Tony Sudbery

Ideas from science fiction often find their way into serious theoretical physics, but one idea which has always seemed unlikely to cross the border between science and fiction is that of a time machine. Nevertheless, this has now happened with the publication in *Physical Review Letters* (**64**, 2965–2968; 1990) of a paper by Yakir Aharonov, Jeeva Anandan, Sandu Popescu and Lev Vaidman which contemplates the prospect of a "quantum time-translation machine".

Time translation is a theoretical concept arising from physicists' habit of treating time and space in the same way. A translation in space is the operation of moving objects in some direction through a given distance. Analogously, a time translation is the imagined operation of moving an event to a different position in time. Until the paper of Aharonov *et al.*, nobody would have supposed that such a translation could be anything other than a purely mental process.

The principle behind their time-translation machine is the fundamental feature of quantum mechanics termed superposition — the combining of different physical situations to give a new situation in which, by the indeterminism inherent in quantum mechanics, either of the original situations might manifest itself. Something of this idea can be grasped by thinking of superposing photographic images, but this does not prepare one for the crucial point that the relative contributions of the two original situations to the superposition are measured by numbers which can be negative.

Part of the mystery of the principle of superposition lies in the fact that the things that are multiplied by (possibly negative) coefficients and then combined algebraically are not measurable quantities but are larger and more abstract: whole situations, histories, states of affairs. Aharonov *et al.* show that in certain conditions this abstract superposition can be reduced to the more familiar arithmetical addition of numerical quantities. They apply this to forces (technically, to hamiltonians) by considering a system in which the force can be set by some external control, and then arranging for a superposition of the situations arising from different settings. This, they show, comes down to simply taking a weighted average of the possible forces. But quantum mechanics, by allowing negative weights, leads to the possibility of a weighted average that lies far outside the range of the individual forces. So a number of small forces can be made to 'average' to a large force.

So far this describes nothing more conceptually startling than a new form of amplification. What raises the possibility of time translation is the very direct relationship between the strength of a force (in the hamiltonian sense) and the length of time it takes to produce a given effect: doubling the hamiltonian has the same net effect as doubling the period of time for which it operates. The use of superposition suggested by Aharonov *et al.* can therefore be applied to periods of times instead of forces. If one could superpose a number of situations in which different periods of time have elapsed, one could, by suitably choosing the weights in the superpositions, obtain the effect of a lapse of time longer than in any of the original situations — thus taking the system into the future — or even of a negative time, so taking it into the past.

But how can one produce situations, all co-existing at *one* time, in which a force has been allowed to operate for *different* times? To achieve this, Aharonov *et al.* appeal to the general theory of relativity, according to which the time that elapses in a particular place, as measured over a given period of time by an observer in a different place, depends on the gravitational potential in the first place. Thus one could conceivably control the lapse of time by controlling the gravitational potential. For example, Aharonov *et al.* suggest that this can be done by enclosing the system in a massive spherical shell and then altering the radius of the shell.

Aharonov *et al.* have developed the first of these ideas into what they claim will be a practical method of amplification. But there are severe difficulties in the way of using the second idea to construct an actual time-translation machine. First, as Aharonov *et al.* acknowledge, the machine can in principle work only very rarely, as it relies on the random, indeterminate processes of quantum mechanics to produce the requisite state of superposition. Second, states of superposition are delicate affairs, requiring precise and intricate coherence between different parts of a system; they have never been produced in macroscopic apparatus such as Aharonov *et al.* describe. Finally, there are notoriously two ways in which quantum systems develop in time. There is the natural development which happens if the system is left undisturbed, and there are the quantum jumps which occur at a measurement, representing the inevitable disturbance caused by observation. The proposed machine takes the system into what would be the future (or the past) if only the first of these was operating; this is not the same as the actual future or past. So don't hold your breath waiting to meet H.G. Wells's Eloi and Morlocks. ☐

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CELL PHYSIOLOGY

Messenger gets the green light

Robin Irvine

Now that inositol 1,4,5-trisphosphate (InsP$_3$) has been established as a second messenger in animal cells[1], relaying hormonal signals received at the cell surface to intracellular compartments, an exciting question pervades the world of plant physiology — do plants (especially higher plants) also use InsP$_3$ in this way? This is by no means a foregone conclusion, especially if one considers the *débacle* of cyclic AMP, which in the late 1960s was assumed (on a mass of spurious flawed evidence) to be the mediator of many, even most, plant hormone actions. Two papers in this issue[2,3] take a positive step towards placing InsP$_3$ in higher plants on a much firmer footing. They show that if chemically inactivated, or 'caged', InsP$_3$ is microinjected into stomatal guard cells of two plants, *Commelina communis*[2] and *Vicia faba*[3], then when the InsP$_3$ is liberated by a laser flash the intracellular calcium increases[2], the cells go through their physiological function of stomatal closure[2], and potassium channels (known to be controlled by Ca^{2+}) are reversibly inactivated[3]. Gilroy *et al.*[2] also show that releasing 'caged' Ca^{2+} inside the cells in a similar way induces the same effects.

So, assuming that the same cells produce InsP$_3$ when exposed to a natural regulator such as abscissic acid, it is highly likely that InsP$_3$ is indeed an intracellular messenger controlling stomatal closure. We can probably assume that it acts on a specific receptor of the same ilk as that found in animal cells[4], an assumption reinforced by the recent demonstration[5] that vacuolar membranes from red beet have a Ca^{2+}-carrying channel highly sensitive to micromolar quantities of InsP$_3$. Taken together[2,3,5], these data make the concept of InsP$_3$ as a second messenger in higher plants a comfortable idea to live with.

However, the stomatal response[2,3] is a rapid one that occurs over only a few minutes — not exactly racing by mammalian standards admittedly, but when viewed against the life cycle of a bristlecone pine this is definitely the fast end of the timescale. These observations therefore add a new urgency to the question of whether plant hormone responses of the more conventional sort (hours to days, and upwards) also use the inositide signalling system. There, things are much less certain.

First, detecting a hormonally-induced response has been extremely difficult. To date, there is no unequivocal demonstration of increased InsP$_3$ formation caused by a natural stimulus in higher plants. Plants, unfortunately, show a much greater ingenuity than animals when it comes to a question of what to do with inositol[6]. Feeding tritiated *myo*-inositol to plant tissues does indeed result in some radiolabelling of phosphatidylinositol 4,5-bisphosphate, PtdIns(4,5)P$_2$ (refs 7,8), the precursor of InsP$_3$, but it is present only in extremely small concentrations, and the analysis is complicated by the presence of other lipids that incorporate inositol, look like PtdIns(4,5)P$_2$ on thin-layer chromatograms, but are other things entirely[9]. (But note that PtdIns(4)P, the precursor of PtdIns(4,5)P$_2$, is the lipid which in cultured tomato cells turns over the fastest[9].) The water-soluble components of [^{3}H]*myo*-inositol-labelled tissues show a similar complexity[10], and many of the radioactive anionic compounds detected are not inositol phosphates at all. Against this alarming background of new knowledge, earlier claims that high concentrations of PtdIns(4,5)P$_2$ (similar to those in animals) exist in higher plant tissues, and that growth-promoting hormones stimulate the formation of InsP$_3$ (ref. 11 and references therein) must be viewed with some caution. In short, although higher plants do have the necessary enzymatic machinery to make and respond to InsP$_3$, we still have little idea what they use it for (apart from controlling stomatal closure).

In animal cells, all but the most acute responses (less than a minute) mediated by inositides involve second messengers in addition to InsP$_3$. So, in view of the fact that we have evidence for the involvement of InsP$_3$ only in rapid plant responses[2,3,12], it is relevant to ask whether plants also use these other messengers. For example, an animal cell needs to stimulate the entry of Ca^{2+} from the extracellular milieu to keep its Ca^{2+} concentration high for anything longer than necessary for a transient response.

As the mist surrounding the probable role of inositol 1,3,4,5-tetrakisphosphate (InsP$_4$) in controlling the entry of Ca^{2+} in animal cells begins to clear a little[1,13], it is pertinent to ask whether plants can also make this compound. *Chlamydomonas eugametos* has a very active and specific InsP$_3$-3-kinase (L. A. Stephens, A. Letcher, A. Musgrave and myself, unpublished data), and cell-free extracts of pea roots can phosphorylate InsP$_3$ into a substance which is probably InsP$_4$ (J. Chattaway and B. Drøbak, personal communication). But *Chlamydomonas* is a lower plant containing amounts of PtdIns(4,5)P$_2$ similar to those found in animal cells[7], and the putative InsP$_3$-kinase activity in pea roots is as yet incompletely characterized, so this question is still open. Although Ca^{2+} influx has been demonstrated in stomatal guard cells, evidence for hormonal stimulation remains elusive[14]. It is also true that the vacuole in the average vacuolated cell probably contains enough Ca^{2+} to keep the cytoplasm supplied for years, so if that organelle is indeed the target for $InsP_3$ (refs 5,15), then one could certainly question whether higher-plant cells would always need a Ca^{2+}-entry mechanism.

The role of diacyglycerol as a second messenger in higher plants (to stimulate protein kinase C) is similarly unclear. There are no convincing reports of a diacylglycerol-stimulated protein kinase, and although a search of two higher-plant genomes for something resembling protein kinase C produced homologues[16], they are considered markedly different and are much more likely to be regulated by something other than diacyglycerol: lysophospholipids are one possibility[17]. Thus, out of the three second messengers which in animals cells mediate the 'classic' phosphoinositide response — $InsP_3$, $InsP_4$ and diacylglycerol — the role of the last two in plants is uncertain.

In conclusion, even if we take it that higher plants do indeed use $InsP_3$ to modulate rapid responses, we remain in frustrating ignorance about its place, and the place of the phosphoinositide cycle in general, in the physiology of higher plants. So the short answer to the question of whether higher plants use $InsP_3$ as a second messenger seems to be yes — but for how long? ☐

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MANTLE CONVECTION

Deep causes of hotspots

Marcia McNutt

Of the three principal types of volcanoes on the Earth's surface, those at mid-ocean ridges and island arcs are relatively well understood as melting processes triggered by the relative motion of tectonic plates in divergent and convergent zones, respectively. Hotspot volcanism, which produces linear chains of volcanoes (the Hawaiian Islands, for example) as the plates drift over melt 'plumes' fixed with respect to the deep mantle, is not explained by the theory of plate tectonics and thus represents one of the few geological phenomena on the Earth's surface that directly provide information on processes in the convecting mantle. In a new compilation of a mass of geophysical data[1], Sleep has deduced the flux of material driving the activity of 37 hotspots, yielding new insights into their global effect.

Sleep's method for calculating the characteristics of each plume depends on its location. For hotspots that have erupted on thickened lithosphere away from mid-ocean ridges, he calculates the total amount of buoyant plume material from the cross-sectional area of the swell that develops in the topography and geoid (height of the Earth's gravitational equipotential surface) as hot material is injected into a thin low-viscosity channel at the base of the plume. The mass flux from the plume is independently estimated from the shape of the swell upstream from the direction of plate motion, assuming that it represents the stagnation point of radial flow from the plume interacting with the mantle flow induced by drag of the lithospheric plate. The excess temperature of the plume can then be calculated given its buoyancy and mass fluxes.

For hotspots near mid-ocean ridges, all of the plume flux is assumed to rise to sufficiently shallow depths to cause melting. The mass flux is thus derived from the amount of excess volcanism, as revealed by the topography and crustal thickness.

From the excess temperature of the plumes relative to the surrounding mantle, typically 200 °C, Sleep tries to constrain values of the temperature contrast across the core–mantle boundary, from which he assumes the plume heat flux emanates. He argues that the usual lower bound for this contrast, 1,000 °C (as derived from experimental melting curves for iron[2]), is not possible given his geophysical data. Furthermore, using his estimate that hotspots cool the Earth's core at a rate of 50 °C per thousand million years, Sleep suggests that the temperature contrast at the core–mantle boundary has been sufficient to drive plume-type activity only over the past 1,300 million years. But both conclusions depend critically on the character of the core–mantle boundary. If it comprises both a thermal and a chemical boundary layer, the latter of which seems necessary to reconcile discrepancies between seismic and gravitational studies of the core-mantle topography, Sleep's temperature contrast could apply solely to that across the thermal boundary layer at the base of the convecting mantle from which he assumes the plumes arise. The deeper chemical boundary layer serves to insulate

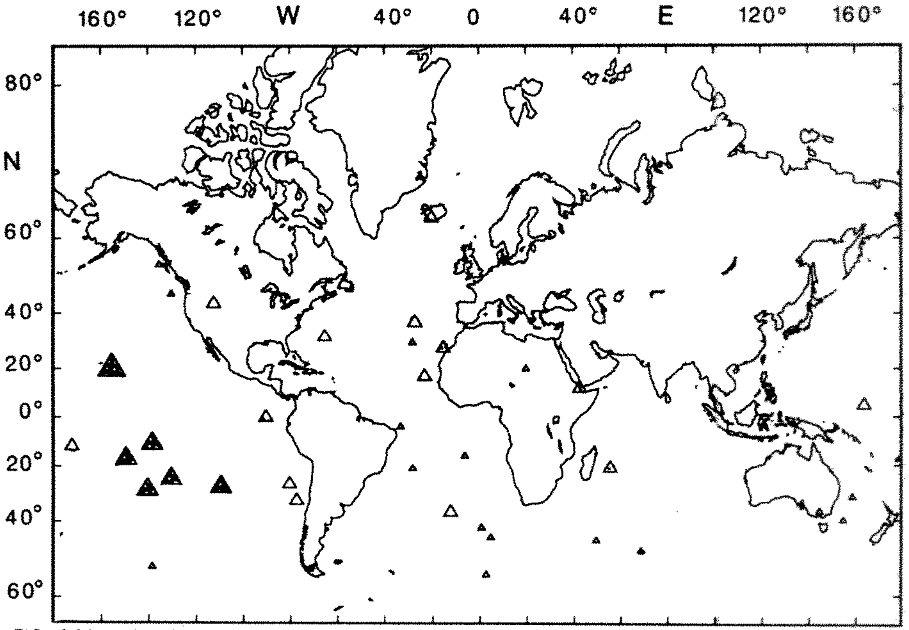

FIG. 1 Map showing the location of hotspots for which the buoyancy flux has been calculated[1]. Buoyancy flux is defined as $\varrho_m \alpha \Delta T Q$, in which ϱ_m is the density of the mantle, α is the coefficient of thermal expansion, ΔT is the average excess temperature of the plume (about 200 °C), and Q is the volume of hotspot material supplied per unit time. Symbol size proportional to the relative magnitude of the flux. Shaded symbols, Hawaiian and Superswell hotspots.

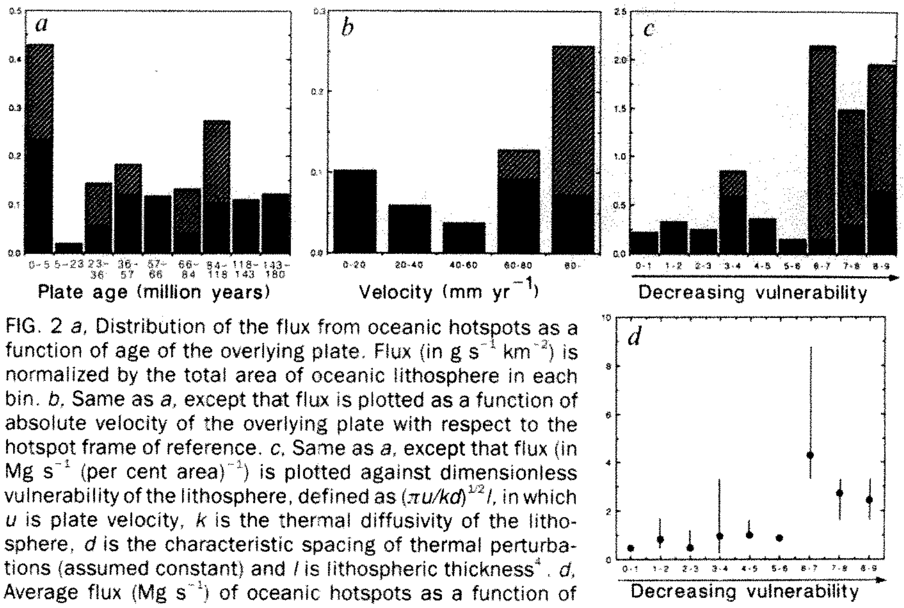

FIG. 2 *a*, Distribution of the flux from oceanic hotspots as a function of age of the overlying plate. Flux (in g s^{-1} km^{-2}) is normalized by the total area of oceanic lithosphere in each bin. *b*, Same as *a*, except that flux is plotted as a function of absolute velocity of the overlying plate with respect to the hotspot frame of reference. *c*, Same as *a*, except that flux (in Mg s^{-1} (per cent area)$^{-1}$) is plotted against dimensionless vulnerability of the lithosphere, defined as $(\pi u/kd)^{1/2}l$, in which *u* is plate velocity, *k* is the thermal diffusivity of the lithosphere, *d* is the characteristic spacing of thermal perturbations (assumed constant) and *l* is lithospheric thickness[4]. *d*, Average flux (Mg s^{-1}) of oceanic hotspots as a function of vulnerability. Vertical bars show the total range of values in each vulnerability category. (Shaded bars, flux from Hawaiian and Superswell hotspots; black, flux from all other hotspots.)

the core from the mantle, thus allowing the core to be much hotter, in agreement with the iron-melting data.

Sleep also concludes that most of the heat flux from mantle plumes is emplaced into the lower oceanic lithosphere, which is subsequently subducted. Thus the surprising result is that mantle plumes, via a circuitous route through the lithosphere, effect a transfer of heat from the Earth's core to the mantle.

Viewed in another way, Sleep's flux estimates can be used to settle the decade-old controversy of whether the non-random distribution of hotspots on the Earth's surface (Fig. 1) represents the fundamental structure of plume-type upwelling in the mantle[3], or the ease with which the overlying lithosphere can be penetrated by melt[4]. Proponents of the first view point have demonstrated that most hotspots are located beneath long-wavelength highs of the geoid, whereas plume-free regions correlate with long-wavelength geoid lows. This global pattern of geoid highs and lows is thought to reflect broad upwelling and downwelling cells in the convecting mantle, which presumably encourage and discourage, respectively, the ascent of plumes from the deep mantle.

Proponents of the lithospheric-control viewpoint have argued that the distribution of hotspots is controlled by the ease with which rising melt from the mantle can penetrate the overlying, cold, lithospheric lid, and thus provides little information on mantle circulation. Because the thickness of the lithosphere increases as the square root of plate age, older lithosphere should be less vulnerable to hotspot volcanism than younger lithosphere. Furthermore, a plate that drifts slowly with respect to the plume source should be more easily penetrated than one that quickly sweeps past it, allowing little time for transfer of heat and melt. But judging solely on the distribution of hotspots, it is difficult to choose between the mantle- and lithospheric-control hypotheses, because there are exceptions to the predictions of both models (hotspots located on geoid lows or erupting on old, rapidly drifting plates). With Sleep's quantification of hotspot flux, however, it is possible to assess whether the exceptions to each rule are significant in terms of the mass and heat carried by hotspots.

Figure 2 shows histograms of hotspot flux as a function of various parameters likely to represent the lithosphere's ability to act as a barrier of hotspot penetration. Because Sleep's hotspot compilation omits some examples on continental lithosphere (on account of difficulties in analysing the relevant data), I have concentrated on the flux from just the oceanic hotspots and normalized the flux with respect to the area of oceanic lithosphere represented in each histogram category. The flux from plumes is discharged without regard to the age of the overlying plate (Fig. 2*a*), except for the two youngest age bins which display an excess of flux near spreading ridges (less than 5 million years old) and a compensating deficit on relatively young lithosphere, found slightly further from spreading centres. As Sleep suggests, this could be either the result of the capture of a nearby ridge by a plume or the sign of entrainment of the flux from a nearby plume into the rising flux at the ridge.

The flux liberated as a function of velocity of the plate also shows no tendency for rapid plate motion to inhibit the flux from hotspots (Fig. 2*b*). Plotting the flux as a function of the 'lithospheric vulnerability parameter', which increases linearly with plate thickness and as the square root of plate velocity[4], again shows that most of the hotspot flux penetrates lithosphere that is predicted to have low vulnerability for volcanism (Fig. 2*c*). The one parameter that does appear to be sensitive to plate vulnerability is the average flux of the hotspots (Fig. 2*d*). Feeble hotspots simply do not occur on lithosphere with low vulnerability (large thickness and/or rapid drift velocity). On the other hand, 85 per cent of the hotspot flux in Sleep's compilation is correlated with positive values of the geoid when referenced to the best-fitting ellipsoid (but 70 per cent when referenced to the hydrostatic figure).

In fact, the impression from Fig. 2*c* is that lithosphere of low-vulnerability, by virtue of its rapid motion (Fig. 2*b*) rather than old age (Fig. 2*a*), is, ironically, the site for most of the global flux of hotspot volcanism. This conclusion is entirely dictated by the large flux from Hawaii and the Superswell[5] hotspots of French Polynesia, which together account for 45 per cent of the global flux. This correspondence between rapid plate motion and large plume flux holds not only for the present, but also for a previous period of rapid plate motion 70–100 million years ago when massive volcanism from Superswell hotspots produced the Darwin Rise seamount chains now located in the northwest Pacific[6]. If indeed mantle circulation (as indicated by the global geoid pattern) controls the distribution of plume flux, then the large discharge of plume flux through rapidly drifting plates with low vulnerability may point to a connection between rapid plate motion and mantle upwelling.

This link between hotspots and plate speed is quite distinct from earlier suggestions[7] that plumes must be part of a broad flow of mantle driven by plate motions. This idea arose because hotspots cluster near mid-ocean ridges and avoid subduction zones. I now doubt the evidence used to support this new theory. The analysis[1] demonstrates that the ridge-related entrainment of flux is only a local phenomena (Fig. 2*a*; 23 million years in time is equivalent to 1,000 km of movement), and plumes near subduction zones might easily be mistaken for island-arc type volcanism. Indeed, some arc systems show isotopic and trace element signatures derived from a plume source[8]. ☐

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HUMAN GENETICS

Neurofibromatosis gene cloned

Bruce Ponder

ANOTHER high-profile human disease has fallen to the strategy of genetic linkage followed by detailed mapping and cloning of the gene[1-3]. This time it is the gene for type-1 (Von Recklinghausen) neurofibromatosis (NF-1), a disease that affects an estimated 1 in 3,500 of the general population. This type of neurofibromatosis is the autosomal dominant disorder popularly (though erroneously) associated with Joseph Merrick, the 'Elephant man'. Sequence homology suggests that the *NF-1* gene product shares similarities with the mammalian GAP gene[4] (see Wigler's News and Views article on page 696 of this issue). The widespread effects of the *NF-1* mutation suggest that its biology will be interesting. Moreover, the gene has already provided a surprise by having embedded within its introns several other genes, transcribed in the opposite orientation, a feature previously described in man only in the factor VIII gene[5].

NF-1 primarily affects tissues derived from the neural crest (see table)[6,7]. Variability is a prominent feature. The biology is a puzzle — it is not even known which cell types are primarily affected. For example, in the common skin neurofibromas (see figure, overleaf) the histological appearances suggest a disordered interaction between axons and the surrounding Schwann cells. If so, the primary defect could lie in either cell type, or in both. The origin of the tumours is not understood either. Both histology and X-chromosome inactivation studies indicate that neurofibromas are polyclonal, suggesting a mechanism perhaps involving local cellular interaction: but the presence of a primary neoplastic clone within the mixed population cannot be excluded.

The *NF-1* gene was mapped to the long arm of chromosome 17 in 1987. Later, two chromosome translocations involving 17 (t(1;17) and t(17;22)) were found in two NF-1 individuals: pulsed-field gel electrophoresis showed that the translocation breakpoints lie only about 60 kilobases apart, and so it seemed the *NF-1* gene had been tightly localized. It took much longer than expected to find the gene, because although several candidates expressed in nervous tissue were quickly found, they were all rather small and none could be shown to be mutated in NF-1 patients. This is not what was expected: the very high mutation rate in NF-1 (about 1 in 10^4; almost 50 per cent of patients have new mutations) suggested either that the gene would be very large, or that there would be frequent DNA rearrangements indicating the location of the gene.

SOME FEATURES OF NF-1

	Approximate % of gene carriers affected	Probable cell of origin	Comments
Diagnostic features			
Café-au-lait spots (more than 5)	>95	Melanocyte	1 cm or larger pigmented skin patches.
Skin neurofibromas (up to 500 or more)	>90	? Schwann cell (Multiple cell types)	Develop at puberty. Non-malignant. Contain Schwann cells, axons, fibroblasts, mast cells, perineural cells and blood vessels.
Lisch nodules	>90	?	Nodular developmental abnormalities of iris.
Less common problems			
Learning difficulties	30	?	
Short stature	15	?	less than third centile.
Large head	25	?	more than 97th centile.
Skeletal abnormalities:			
Scoliosis	30	?	
Pseudoarthrosis	3	?	Failure of development of one segment of a long bone (usually tibia).
Epileptic fits	<5	?	
Tumours			
Plexiform neurofibromas	20	? Schwann cell (multicellular like skin neurofibromas)	Can cause severe deformity; may become malignant; May be associated with local hypertrophy.
Neurofibromas of spinal and peripheral nerves*	5	As above	
Schwannomas of peripheral nerves	<5	Schwann cell	Occasionally become malignant.
CNS tumours (glioma, ependymoma, occasional meningioma)	3	Glia, ependyma, arachnoid	Optic nerve gliomas relatively frequent often nonprogressive.
Phaeochromocytoma	1	Chromaffin cell of adrenal medulla	Usually benign.
Neurofibrosarcomas; malignant Schwannoma	2	? Schwann cell	Malignant: usually develop from plexiform NF or Schwannoma.

* These tumours are prominent in some families with a variant form of *NF-1* which maps to the *NF-1* locus.

The finding of the present candidate *NF-1* gene illustrates the value of a detailed DNA map of the candidate region, but also the importance of the single rare case with an informative mutation. The Michigan group[1] screened complementary DNA (cDNA) libraries with a conserved sequence found by chromosome jumping, and also with an entire yeast artificial chromosome (YAC) clone spanning the translocation region. The group found two clones covering a sequence of 2,012 base pairs with a single open reading frame containing six exons, and spanning at least 33 kilobases of genomic DNA. Northern blots indicate hybridization with messenger RNA (mRNA) of roughly 13 kilobases. The evidence that this clone is part of the *NF-1* gene is first, that studies in human–rodent hybrids containing the isolated (1;17) and (17;22) translocations show that both the gene sequence and its transcript are disrupted by the (17;22) breakpoint, and second, that one NF-1 patient out of 35 studied had a novel 500-base-pair insertion within or close to an exon of the candidate sequence, absent from his parents' DNA.

The Salt Lake City group[2,3] found that 3 out of 54 NF-1 patients had germ-line deletions in the translocation region. The smallest deletion, of 11 kilobases, did not include any of the previous gene candidates but did include a conserved sequence spanning the (17;22) breakpoint. Using the conserved sequence to screen cDNA libraries, this group first obtained 4 kilobases of overlapping DNA sequence that contained a single open reading frame of about 3,300 base pairs, oriented 5′ to 3′ in the centromere-to-telomere direction. This corresponds to nine exons spaced over a 100-kilobase genomic region extending 65 kilobases from the (17;22) breakpoint in the direction of the telomere.

The evidence that this was part of the *NF-1* gene was first that the 11-kilobase *NF-1* genomic deletion included this gene but not the other candidates; second that the gene was interrupted by the (17;22) breakpoint; and finally, a search for alterations in DNA sequence in exons 4–9 of the candidate gene using single-strand conformation polymorphism[9] revealed six variant alleles in 72 NF-1 individuals and none in 65 controls (although two other

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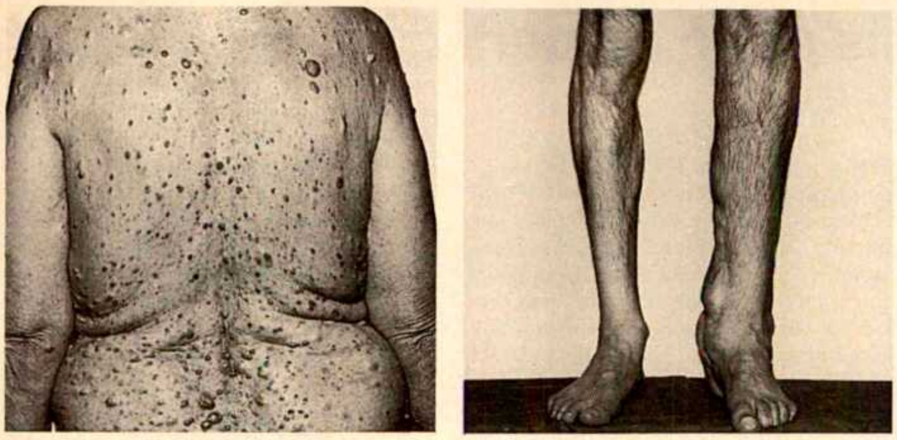

Neurofibromas in NF-1. Left, typical appearance of skin neurofibromas; right, plexiform neurofibroma is present in the lower left leg. Note the local overgrowth of the tissue; the leg is also longer. Plexiform neurofibromas can also be associated with thickening, pigmentation and excessive hair growth of the overlying skin. Photographs from ref. 8.

non-NF-1 individuals did have variants). The candidate *NF-1* gene sequences identified by each group overlap. Subsequently, the Salt Lake City group obtained sufficient additional sequence information to reveal significant homologies between the NF-1 protein and mammalian GAP (ref. 4).

The gene is expressed in a wide variety of tissues, including many not of neural-crest origin: the specificity of the NF-1 mutation has therefore to be explained, but might involve tissue-specific modification or interaction of the *NF-1* gene product with other proteins.

Each of the NF-1 mutations described so far is expected to result in inactivation of the gene. Loss of the remaining allele by somatic mutation, as in retinoblastoma (*Rb*) is a possible mechanism of tumour formation; but it seems less plausible to account for more general manifestations such as short stature or learning difficulties. Dosage effects from loss of one copy seem more likely. More complicated (and speculative) schemes involve possible effects on the activity of the *NF-1* gene of transcription of the genes embedded in reverse orientation within its introns, either by steric hindrance or formation of RNA duplexes with the *NF-1* gene product, which would be degraded. It is not even clear whether the *NF-1* gene acts as an *Rb*-like tumour suppressor gene in relation to tumour formation. The predicted allele losses in the *NF-1* region in tumours have not been reported so far; but there could be selection for a gene near *NF-1* being retained in the tumours, explaining why the large-scale chromosomal events detectable by the allele loss assay[10] might be rare. Detailed analysis of both alleles of the *NF-1* gene and of transcripts in populations of cells purified from tumours will be needed to resolve this question.

The practical, clinical consequences of the cloning are uncertain. Because of the high mutation rate, many pedigrees contain only one NF-1 individual, so prenatal diagnosis using linked markers is impossible. Identification of the mutations will allow prenatal diagnosis, but the practicalities of this will depend on whether most mutations occur in a limited region of the gene. So far, examination of about 10 per cent of the coding sequence in 72 individuals has revealed 6 mutations, spread over 4 exons[3].

Because many cases of NF-1 are so mild, for most people the most important prediction is likely to be not the inheritance but rather the severity of the disease. Some uncommon variants of NF do seem to run true in families[6]; for these, correlation with the type of germ-line mutation seems likely (and could be informative in terms of gene function). Most often, however, it seems that neither the individual features of NF-1 listed in the table, nor overall severity, breed true; the relative contribution to phenotype of germ-line mutation, modifying genes, environment and chance is still unclear. It will be interesting to see whether correlations can be established between types of germ-line mutation of the *NF-1* gene itself or the genes embedded within it and the presence of specific NF-1 phenotypes in families; and whether elucidation of the *NF-1* gene product and its action will provide clues to factors which modify its effects. ☐

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DAEDALUS

High charges

LAST week Daedalus presented his 'ionic windmill'. A big wire grid with many discharge points emits electrons into the air, which form molecular ions with the air. These are blown downwind to a collector grid, whose return current to the emitter captures much of the wind's energy. He now plans to run the whole scheme in reverse, to create an ionic helicopter.

This elegant craft simply consists of two parallel horizontal wire meshes. The upper, negative mesh will be studded with downward-pointing sharp points coated with material of low work function. The ion emission from these points will be attracted towards the lower, positive mesh. Ions diffuse only slowly in air, so their downward flow will entrain a strong downdraught: the well-known 'electric-wind' phenomenon. The voltage to drive the craft will come via suitable inverter electronics from an array of solar panels mounted above the top grid: on edge, so as not to impede the downward airflow, and spaced so as not to shadow each other.

A structure of two open meshes braced to rigidity by insulated struts between them, and powered by solar panels, is already lighter and stronger than any other airframe. The 'ionicopter' should be wonderfully efficient as well. To minimize the kinetic energy wasted by its down draught, a helicopter must push down the maximum volume of air at the minimum velocity. A wide wire-grid structure can capture far more air than any rotor, and can accelerate it gently and silently without wasteful turbulence.

The ionicopter will make an ideal aerospace vehicle. At take-off, a small outward-facing corona-discharge point will eject positive ions till the whole craft is negative enough to initiate downward electric-wind discharge from the upper grid. The potential difference between the grids will then be increased until the downdraught lifts the vehicle into the sky. Thereafter, the higher it goes, the better. Its Sun power becomes more reliable, the solar cells gain efficiency in the cold upper air, the electric discharge becomes more copious at low pressures, and the velocity of the downdraught rises to compensate for its falling density. Unlike any winged aircraft, it can soar almost into space. Given batteries to store surplus solar energy for use at night, it could stay up indefinitely.

Daedalus's ionicopter will compete strongly with satellites as a research vehicle, a TV relay and photoreconnaissance platform. At night, it will be visible from the ground by its auroral glow-discharges in the low pressure of aerospace. The ozone they generate will go to replenish the halocarbon-sabotaged ozone layer.

David Jones

Whale migration record

SIR—We recently confirmed a new long-distance migration record for mammals (apart from *Homo sapiens*). We found that a humpback whale (*Megaptera novaeangliae*) has migrated from the Antarctic peninsula region (64° 20′S; 62° 27′W) to Colombia (2° 57′N; 78° 12′W). The shortest swimming distance between these locations is more than 8,334 kilometres. We identified the whale at both locations using photographs of the distinctive ventral pigmentation on the tail flukes[1]. The whale was seen in Antarctica on 19 April and in Colombia on 28 August 1986. This is the first time that a humpback whale has been shown to cross the equator, and the first time an Antarctic humpback whale has been documented in South American waters. This finding has interesting implications for the possibility of migration between hemispheres.

The match of pigmentation was found by comparing a catalogue of 32 humpbacks identified from the Antarctic peninsula[2] and a collection of 86 identified off the coast of Colombia. The whale swam from Antarctica to Colombia in less than 5 months. Following the typical humpback migration strategy of spending summer in high latitudes and winter in low latitudes[3], this whale probably belongs to a population that feeds in Antarctica and breeds off the coast of Colombia.

It is also possible that Antarctic peninsula humpback whales migrate up the eastern side of South America to the coast of Brazil, where there is another breeding ground. The swimming distance from the Antarctic peninsula to this area is shorter by more than 1,800 kilometres than to the Colombian site. This raises the interesting possibility that the Antarctic peninsula feeding grounds may be an area from which humpback whales can cross between the South Pacific Ocean and the South Atlantic Ocean. It is of interest to note that only 1,000 kilometres further north of our Colombian study site there is a site used during the breeding season by humpbacks that feed in the Northern Hemisphere (G. H. Steiger, J. Calambokidis and R. Sears, unpublished data).

Despite opposite migration schedules, it might be possible for an occasional individual from the Southern Hemisphere to breed with members of this Northern Hemisphere population. If this also occurred on the east side of South America, it would offer a mechanism for Baker's supposition[4] that the relatively small genetic distance between the North Pacific and North Atlantic populations could be the result of periodic gene flow between oceans. Although previous research clearly demonstrates that most humpbacks probably do not cross the equator or move between oceans, the possibility that individuals occasionally might do so has important implications for gene flow, population differentiation and population management, and deserves further study.

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Rotating gyros and gravity

SIR—The report by Hayasaka and Takeuchi[1] that the gravitational acceleration of a rotating mass depends on both the magnitude and vectorial direction of the angular momentum suggests several considerations. It is well known[2,3] that the motion of an extended body in the Einstein theory of gravitation may depend on spin and angular momentum: the free motion of an extended mass endowed with angular momentum need not be geodesic. There are several theories of gravitation other than Einstein's in which such effects are definitely predicted. Accordingly, excitement at the report of this effect should focus on its size and asymmetry and implications for Einstein's theory of gravitation rather than on the question of whether it exists.

The possibility that angular momentum may influence the gravitational acceleration of a rotating mass was perhaps first noted in 1912 by Nordström[4] in a paper in which he tried to derive a Lorentz invariant theory of gravitation from a scalar potential. Nordström quoted a communication from Einstein, ending: "A simple example shows that in such a theory a rotating object in a gravitational field will have a smaller acceleration than one which is not rotating." Einstein's arguments were never published, and he never subsequently returned in print to the subject.

Explicit calculations of the effect have been published[5], however. An analogous, extremely elementary calculation for the Einstein equations shows the effect, in contradiction to the results of Papapetrou and Synge, to be (precisely) absent for the case of a small object with the angular momentum vector parallel to the gravitational field.

For the Nordström theory and newtonian theory modified to be compatible with special relativity, the gravitational acceleration of a rotating object is independent of both the mass of the object and the orientation of the angular momentum vector to the field direction. Rather, it depends on (roughly) the average tangential speed in the horizontal plane, when reduction of the acceleration of gravity is of the second order in v/c or about 1 part in 10^{10}. Qualitatively, it seems to be simply related to the fact that acceleration in special relativity depends, *inter alia*, on components of the velocity transverse to the acceleration. Similar effects are suggested in many other theories of gravitation.

The meticulous nature of the report of Hayasaka and Takeuchi allows for further experiments by others. It is to be hoped that the arrangement of equipment in some of these tests would measure the dependence of the effect on the magnitude of the polar angle of the angular momentum vector with respect to the vertical.

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Sunspot cycles and climate

SIR—Geller[1] in News and Views discussed the implications of a report by James and James[2] in which an oscillation of about a decade long appeared in power spectra of certain output from a model of the global troposphere. Because the oscillation was generated internally — that is, within the atmospheric model, both authors concluded that "there is no reason to appeal to external forcings to account for ultra-low-frequency atmospheric variability". This statement is obviously based on the numerous claims made to attribute approximately decadal cycles in climate time-series to forcing by the nominal 11-year sunspot cycle, and that so far, no generally accepted and quantitatively based physical mechanism has been provided to explain a connection between solar and atmospheric variability. But if the new general circulation model simulations are correct, they could be interpreted as indicating that the search[3] for a link between the sunspot cycle and weather should actually be continued.

An important step in studying signals in time-series, once a signal of interest has been identified in the power spectrum, is to generate the waveform in the time domain. This has already been done[4] for the nominal 11-year wave occurring in many surface climate time-series. A similar waveform occurs in a 250-year snow accumulation record from a northern mid-tropospheric site[5]. These waveforms, particularly the latter, exhibit pronounced amplitude modulation and phase shifts with respect to the sunspot cycle, which itself exhibits amplitude as well as frequency modulation. The waveforms derived from climate data may be approximately in phase with the solar cycle for a certain number of cycles only, before passing through a low-amplitude disordered sequence followed by a further group of clear cycles where the phase relationship may be retained or it may switch 180° (ref. 4). With the exception of the latter effect, these observations may be substantially explained by the interaction of two atmospheric waveforms differing in period by about 1 to 2 years[6]. Thus, the solar cycle added to an approximately 10-year wave could produce the observed waveforms.

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Helical period of Z-DNA

Sir—Pui Shing Ho of Oregon State University, and Michael Youngquist and Robert Driscoll of the California Institute of Technology, have independently pointed out that, in our paper "Scanning Tunnelling Microscopy of Z-DNA" (*Nature* **339**, 484–486; 1989), the apparent helical period in our Figs 1 and 2 is considerably longer than the 43 Å generally accepted for Z-DNA.

Both sets of authors have suggested that the 70 × 46 Å boxed area in Fig. 2*a* of our paper (enlarged in Fig. 2*b*) may actually represent nearly two full turns of the helix. In that case, the prominent grooves would probably both be minor grooves corresponding to successive turns of the helix, since the bases in the major groove form a convex surface. This agrees with the observation by Ho that Fig. 1*b* "shows ten peaks and nine valleys between 0 and 400 Å along the length of the fibre and is consistent with a 41.5-Å spacing of each vertical displacement." Youngquist and Driscoll point out that "the molecular electrostatic potential surface has a major groove three times wider than the minor groove; the dark bands across the molecule in the STM image, however, are of nearly identical width", as would be the case for the van der Waals surface. They note that the 4–6-Å width of the dark bands accords well with the 3–5-Å width obtained crystallographically. They also point out, correctly, that the Fourier transform frequencies at 20.4, 14.5 and 7 Å in Fig. 1*c* cannot necessarily be attributed to helical structural features, as they are close to harmonics of the 42-Å period. Such harmonics would be expected since the helix is not a pure sinusoid.

We admit that these suggestions could well be the proper interpretation of our images. We did not notice the discrepancy in dimensions, and were seduced by the excellent visual match between the STM image and the electrostatic model (Fig. 2*c*). The van der Waals surface, while it gives reasonable correspondence with the grooves in Fig. 2*b*, does not show the variations in width perpendicular to the helix axis that are evident in Fig. 2*b*, *c*. On the other hand, if Fig. 2*b* is scaled from 70 to 46 Å and projected on Fig. 2*c*, the fit is remarkably good.

It seems likely that an STM image of DNA could reflect a complex mix of van der Waals and electronic properties. The interpretation of STM images of macromolecules such as DNA involves other issues in addition to the topographical contour, such as the size and structure of the probe tip, its response as it scans along graphite and approaches a thick molecule from the side, its pressure on the sample, and the inhomogenous electronic structure of the polymer. The most dependable information lies in the periodicities, those features which repeat regularly, regardless of the mechanism of image formation. Judged by this criterion, our images clearly show the left-handed helix and helix-repeat characteristics of Z-DNA.

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Nepotism in the honey bee

Sir—Reports that honey-bee *(Apis mellifera)* workers discriminate nepotistically among nestmate patrilines, by Page *et al.*[1] and others (for example, refs 2–8), have generated considerable interest. But Queller *et al.*[9] recently found that *Polistes annularis* wasps, under natural conditions, do not preferentially join more closely related natal nestmates during foundation of their spring colonies. Similarly, within colonies of the polygynous carpenter ant *Camponotus planatus*, workers fail to distinguish their mother queen, sister workers or sister virgin queens from those of other naturally cohabiting nestmate matrilines (N.F.C. and S. Cover, unpublished results). The discrepancy between the results obtained in wasps and ants and in the honey bee could reflect a unique kin-recognition[1] ability of the latter. Alternatively, we suggest that the composition of experimental colonies containing artificially low numbers of patrilines, of artificially high phenotypic distinctiveness, may yield nepotism as an artefact[10].

Apparent kin recognition can easily arise as the accidental by-product of different discrimination mechanisms based on genetically correlated cues (such as species or mate recognition)[11]. Social insects are acutely sensitive to foreign odours normally used to identify and reject non-nestmates from the colony. Honey-bee workers in colonies of artificial phenotypic heterogeneity may perceive their peculiar nestmates as smelling more like foreigners than like ordinary nestmates. Discrimination in such behaviours as worker interactions, swarming and queen brood rearing may result, not from a preference for one's own patriline, but from a bias against the other, though not reaching the threshold necessary for rejection[7,10]. Strange-smelling larvae may in addition elicit discrimination that is normally directed against diseased brood, though again below the threshold for removal; such behaviour is known to be important for disease resistance in honey bees[12].

Various experimental methods have been used to form genetically mixed colonies of honey bees, all of which combine a diversity of phenotypes which may not typically coexist. In early studies, colonies containing multiple kin groups were formed by adopting brood between foreign nests, of known or assumed relatedness[2,3]. An apparent improvement was introduced by artificially inseminating a queen with drone sperm bearing heritable colour markers, yielding half-sibships visually distinguishable to the investigators (for example, refs 4–8). Because bees recognize relatives by olfaction, the colours themselves were usually assumed not to affect recognition responses. But marker genotypes have been shown to alter the bees' odour phenotypes as well. When a queen heterozygous for a recessive marker is inseminated with sperm from a single recessive drone, the resulting two worker groups exhibit some preference for nestmates of their own colour, though all are full sisters[13].

Use of allozyme markers[1] rather than colours does not eliminate the problem without evidence that the variation between genotypes at other loci is comparable to that among offspring of a naturally mated queen. Particularly if the marker-carrying lines used were originally drawn from different populations, they may well differ in a variety of metabolic traits that exaggerate their olfactory discriminability. In addition, the number of patrilines in these experiments has been limited to two or three, the number of genetic markers available. In nature, honey-bee queens reportedly mate with 7–17 males[14], presenting workers with a more daunting discriminative task. One study indicates that the nepotistic behaviour observed in two-patriline colonies disappears in colonies containing seven or eight lines[8].

The degree of discrimination observed within honey-bee colonies is generally weak, never reaching a 2:1 preference for full sisters. Yet workers that are able to distinguish among partrilines should maximize their inclusive fitness by aiding full sisters exclusively, particularly when rearing queen larvae — assuming genotypic equivalence in reproductive value. (Feeble full-sister larvae should not be exclusively preferred to vigorous half-sisters. But variation in brood vigour would never lead multiple worker lines to prefer full sisters; rather, each line should prefer the same larvae.) Selection for the collective efficiency of all lineages has been suggested as a brake on kin-group selfishness within the colony[1], but it is unnecessary to invoke adaptation on the colony level to account for the pattern. Biases are expected to be weak when kin recognition is not itself under selection, but is a by-product of another genetic discrimination system[11]. By contrast, stronger patrilineal differences are reported in studies of genetic influence on honey-bee division of labour, a phenomenon which has also been documented in colonies containing naturally mated queens[15].

Finally, kin recognition can also be induced within ant colonies of artificial phenotypic heterogeneity. When *Camponotus floridanus* workers originating from distant locales (Tallahassee and the Florida Keys) are experimentally adopted into one nest, they detect and persistently investigate nestmates from the other population, with a bias of 1.12:1 (ref. 16). Consistent with the hypothesis that discrimination represents not nepotism, but a side-effect of inter-colony hostility, workers use their recognition ability to bias weak aggression toward alien nestmates, but fail to prefer sisters in cooperative food exchanges and grooming. Given the apparent absence of kin recognition in natural colonies of ants and wasps, and the inconclusiveness of the evidence for honey bees, we would argue that adaptive nepotism among nestmates has still not been demonstrated in any social insect.

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PAGE *ET AL.* REPLY—Carlin and Frumhoff suggest that a reduced number of subfamilies ('partrilines' derived from different fathers) may lead to atypical behaviour, citing the study of Hogendoorn and Velthuis[8] as evidence. Hogendoorn and Velthuis established colonies that had two distinguishable subfamilies, yellow and black, and other colonies that had seven or eight subfamilies, one yellow and six or seven black. They then observed the feeding and 'aggressive biting' interactions of individuals within these colonies to determine if food was being preferentially exchanged among members of the same subfamily and if aggressive biting occurred between individuals of different subfamilies.

We calculated biases directly from the marginal totals of their contingency tables; in all four trials of two subfamilies and three of four trials of seven and eight subfamily colonies, the biases were in the direction expected if preferential kin discrimination was occurring. The bias was statistically significant for one trial ($P < 0.01$) of an eight-subfamily colony while the other three trials lacked sufficient statistical power to test any effect because sample sizes were too small (8, 22 and 3 observations of yellow–yellow worker interactions, respectively). We believe that Carlin and Frumhoff's interpretation of ref. 8 to show a lack of discrimination in worker interactions is incorrect.

The suggestion that the use of genetic markers affects recognition directly or by linkage with genes that affect recognition, is important and plausible. But Frumhoff in his unpublished study[13] did not resolve whether the single, recessive gene marker used, or genes linked to that marker, affected recognition. This kind of linkage effect does not influence the reported kin-recognition studies because the markers and their associated linkage groups are distributed randomly within each subfamily; subfamily composition is determined by the paternal genomes. Linkage is not an issue for subfamily recognition because all paternal genes are linked, a consequence of haplodiploidy.

The real issue associated with genetic markers and kin-recognition studies is whether the markers themselves can affect recognition. Colour and allozyme markers occur naturally and are polymorphic in most populations. Perhaps the most significant argument against Carlin and Frumhoff is that studies using allozymes, single-gene recessive mutations and polygenic integument colour yield the same kind of result as studies that use naturally mated queens and no markers at all[18].

Genetic manipulation may result in greater genotypic diversity in colonies than would occur under natural conditions, though there is no evidence for this in honey bees. Greater phenotypic diversity may increase the experimental resolution of phenomena that occur at very low levels in colonies with less diversity, rather than introduce artefacts. These low-level effects that have been so frequently reported are themselves intriguing evolutionary puzzles.

Experimental evidence does exist to support the conclusion that worker honey bees can recognize and discriminate among nestmates from different subfamilies under experimental conditions. Although everyone acknowledges the limitations of their experiments, there is no evidence that these limitations introduce experimental artefacts.

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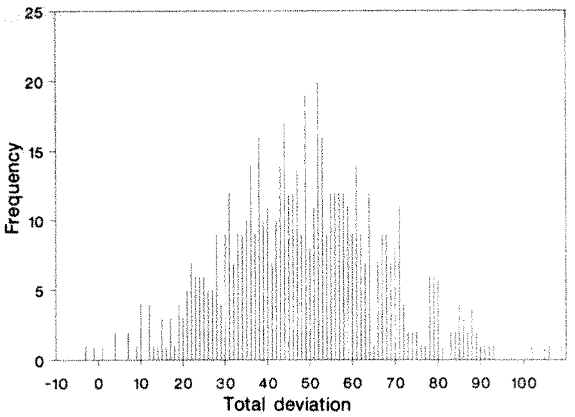
SIR—Page *et al.*[1] recently claimed that honey bees working on queen cells bias the rearing of queens in favour of supersisters rather than half-sisters. To test the statistical significance of the finding, Page *et al.* identified "nepotistic" subfamilies (those with highest ratio of adults on queen cells relative to larvae), and summed deviations between expected queen frequency (based on larval frequency) and observed queen frequency of these subfamilies for 30 trials. Page *et al.* then used a computer simulation to generate a population with subfamily frequencies equal to estimates from pooled experimental larvae and queen frequencies, drawing experiment-sized samples for larvae, queens and adults. The simulated nepotistic subfamily was determined as described, and deviation in frequency of these subfamilies between queens and larvae were calculated for 30 simulated trials and summed. The probability of obtaining simulated total deviations as extreme as that observed was estimated as the per cent of 1,000 simulations in which



Simulation was used to generate 600 data sets each of 3 trials of 10 colonies of 3 subfamilies at equal frequency. The frequency distribution of simulations which produced each deviation in 'observed' and expected numbers of queens for nepotistic subfamilies is plotted. Page *et al.* observed a deviation of 60 queens for their experimental data. The figure shows that this is well within the range expected by chance. Data sets were also generated for colonies with differing subfamily relative frequency. Results obtained were similar to those in figure.

the total deviation exceeded 60.

The simulation performed by Page *et al.* supports the nepotism hypothesis, but this probably stems from the interaction of two experimental and two simulation deficiencies. The experimental deficiencies are the small sample sizes and that 6 of the 10 experimental queens were heterozygous for MDH, necessitating allocation of subfamily affinity on the basis of probability. These shortcomings mean that subfamily frequencies were estimated with high sampling error.

The simulation deficiencies are: first, for the real data, the wrong subfamily will be designated nepotistic when, through sampling error, its relative frequency is underestimated in larvae, causing large deviations between observed and expected frequencies in queens. Incorrect subfamilies will be chosen less often in the simulations, because all genotypes are known, and the sample size is larger. Second, worker and queen samples are pooled to provide estimates of simulated subfamily frequencies. (Expected values for the observed data were calculated from larval frequencies alone.) The weighted average is inevitably intermediate between queen and larval frequencies, thereby reducing the magnitude of deviations between frequencies in the simulated queen and larval samples. Thus the total deviation will appear smaller for the simulated data than the observed. If identical procedures are used for the observed data (queen frequencies are predicted from pooled larval and queen frequencies), then the total number of excess queens is −2, indicating no nepotism.

We generated 600 random 'data' sets drawn from a population of three subfamilies at equal frequency and analysed them using the procedure of Page *et al.* (see figure). Of our data sets, 26 per cent had deviations exceeding 60. We then "estimated the probability of getting a deviation that was as great or greater than our results", using the Page *et al.* simulation. Of our data sets, 84 per cent produced a 'significant' deviation. Thus even data drawn from a totally non-nepotistic population can exhibit 'statistical significance' with these procedures.

There is no evidence in the data of Page *et al.* that subfamily relative frequencies differ significantly between workers and queens, which they should if nepotistic rearing occurred. It is suggested that nepotistic subfamilies are genetically predisposed to rear queens. But nepotistic subfamily identity varies within colonies, suggesting that genetic determination of nepotism does not exist.

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PAGE AND ROBINSON REPLY—The ability of worker honey bees to discriminate among queen larvae on the basis of kinship has been controversial since two papers were published in 1984, when R.E.P. and Erickson reported[19] that workers could distinguish between nestmate and non-nestmate larvae while Breed, Velthuis and G.E.R.[2] failed to find evidence for discrimination. These and other subsequent studies (for example, refs 3, 4) can certainly be criticized on the basis of some artificiality introduced by the experimental methods, criticisms usually stated by the authors themselves (see ref. 17).

Oldroyd *et al.* correctly point out that our demonstration[1] of nepotistic queen rearing is the result of a sampling bias in the Monte-Carlo simulation we used. We have now reanalysed some of our original data using more conventional statistical methods that are free of the biases present in the simulation model. Full details are available on request from R.E.P.

Behavioural heterogeneity within honey-bee colonies is in part a consequence of colony genetic structure. In our study, this was most dramatically demonstrated by the difference in the subfamily composition of the adult workers sampled on queen cells (presumed to be engaged in queen care) and the composition of samples of worker larvae (presumed to be the pool from which larvae were drawn for workers to select among as queens, or remove). This heterogeneity probably results from patterns of sperm use by queens resulting from the incomplete mixing of sperm from their many mates, or from behavioural biases that are a consequence of the genotypes of workers. The effect of genotype is demonstrated by comparing the subfamily representation of adult workers sampled on queen cells versus those sampled on worker brood. Differences in these distributions are significant in 4 of 10 colonies and highly significant for all colonies combined (our unpublished data, available from R.E.P.).

Worker larval samples differ from queen samples, but these differences are only marginally significant. Both queen and worker larval samples fluctuate significantly from trial to trial within colonies, but these fluctuations are different, suggesting that some additional nonrandom process is affecting the subfamily representation of queens. We suggest that this process involves discrimination during queen rearing.

Our results show that honey-bee colonies have a genotypic and behavioural structure that could favour nepotism during queen rearing, provided that larvae are genotypically labelled, and workers have kinship-related information about larval labels. These necessary elements have all been reported elsewhere[17]. But a conclusive demonstration of nepotistic queen rearing remains elusive.

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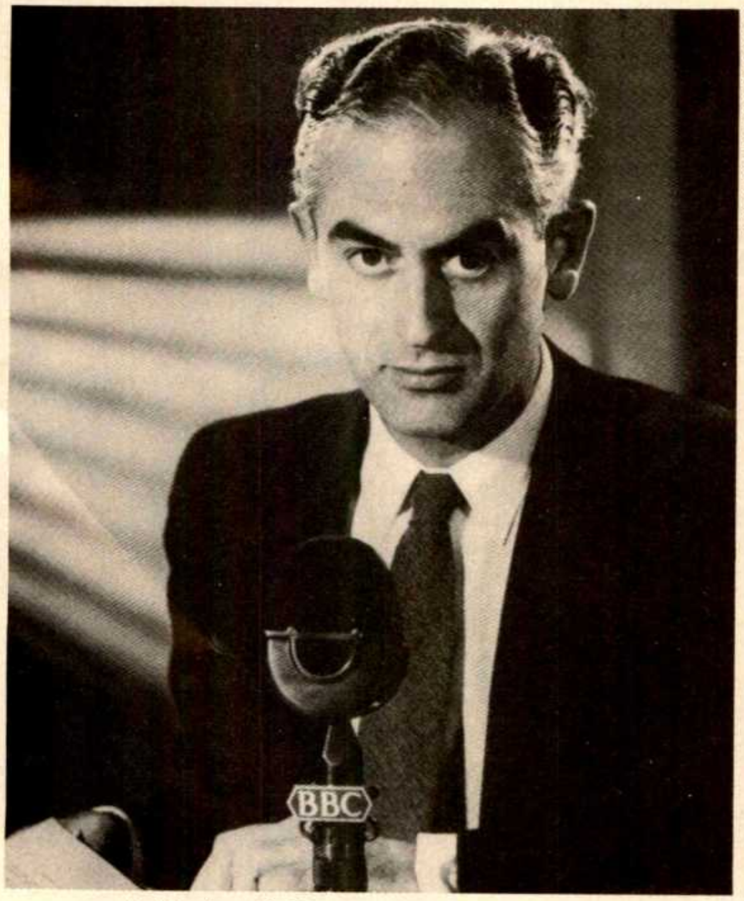
Odder than we can think

Alex Comfort

The Threat and The Glory. By Peter Medawar. *Oxford University Press: 1990. Pp.291. £15. Published in the United States by Harper Collins, $22.50.* **A Very Decided Preference.** By Jean Medawar. *Oxford University Press: 1990. Pp.256. £15. Published in the United States by Norton, $19.95.*

ON AN afternoon in the 1960s, Macfarlane Burnet gave a lecture at the British National Institute for Medical Research chaired by the director, Sir Peter Medawar. The lecture was brilliant but discursive — and long. When Medawar rose to thank the speaker he expressed his appreciation, and proceeded to summarize the lecture in five minutes flat. There will be many who had the privilege of knowing and working with Medawar who will recognize in that incident the mixture of precision and quiet irreverence which made him such a notable communicator of science. He once pointed out that compared with artistic creators, scientific discoverers are expendable because their insights are perpetually superseded, and it is for that reason that as his discoveries, which laid the foundations for transplantation surgery, recede into the history of science, his general and philosophical writings come to the fore. *The Threat and the Glory*, published today, together with a new biography, contains both his Reith lectures on the future of man (when the title was proposed to him, he told us he would lecture on the future of man and the inadequacy of academic salaries) and several essays originally written as reviews. Collections of reviews often fail to work, but Medawar never reviewed anything without producing a polished essay which, like Macaulay's reviews, often had a higher durability than the work which occasioned it.


From A Very Decided Preference.

"Isn't he beautiful?" — attracting students to science.

Medawar the researcher was comprehensively recognized in his lifetime, though the concentration on his immunological work sometimes obscures his scope — he was a fundamental theoretician, for instance, on the nature of ageing. Medawar the philosopher of science is now best known for his championship of Karl Popper and of Whewell, and for his summary exposure of the pretence that science proceeds by induction, which would be "intellectually disabling if it were true". Few eminent biologists of Medawar's time were as philosophically literate: called away from interviewing a raw candidate who wanted to work with him on the nature of ageing, he would ask the victim to glance at a new book by Bradley and, on resuming the interview, inquire what he thought of it. Medawar's demolition of Teilhard de Chardin, not so much for what he said as for the uncritical way he said it, is a polemical classic.

Herein lies something of a mystery, which none of the published essays solves. As a philosopher, Medawar addresses many of the ethical problems of science — notably the gung-ho baconian view that science exists to do anything which is possible because it is possible, an idiocy which represents the "threat" mentioned in the title. Medawar's position is one of liberal humanism, which represents the "glory". Much of his work was concerned with science as the delimiter of the imagination in the light of the reality principle: hence his antipathy to mystics such as Teilhard who fail in attempting to square their mysticism with science, and to the "lava flow of ad hoc explanations" which disfigure freudian psychoanalysis. He enjoyed Thomas Reid, the slightly Alf Garnett-like father of "commonsensism", but he also studied Kant.

Yet his final public posture, which differs little superficially from that of T. H. Huxley, leaves a feeling in the reader of a step not taken: never more, perhaps, from a man whose interest in philosophy was kindled by reading Whitehead, than in the fact that nowhere in his writings does Medawar address the philosophical issues now raised in the laboratory by quantum mechanics. Niels Bohr said that anybody, any philosopher of nature in particular, who was not shocked by this subject had failed to understand it. As a serious mathematician, Medawar clearly understood. Probably, with the awful example of Teilhard, an acute awareness of the imaginative human need to become "bunkrapt" and the obligation of limiting this tendency by science, he was still looking for the right words. One of the profound losses inherent in Medawar's death is that one cannot now discuss this area with him and hear how he would interpret its application to the philosophy of science, and to biology in particular. It was left to his prickly and equally rationalist colleague J. B. S. Haldane to suggest that "nature is not only odder than we think, but odder than we can think".

It was said of Reid that his life gave little occasion for biography. Medawar suffered a severe stroke at the age of 54 yet fought back to resume his work (much as did Louis Pasteur), and despite a devastating series of recurrences and the loss of an eye to glaucoma. This is an epic of personal endurance which he wryly summarized as "a very decided preference" for staying alive.

One of Medawar's earliest encounters with Jean Taylor, his future wife, led him to offer to give her tutorials in philosophy. Everyone who knew the Medawars will confirm that her assessment of her own role in the book she has written about their life together, that it was "limited to enabling", understates her contribution. Despite Medawar's intellectual brilliance and his impressive appearance ("isn't he beautiful?" said a visiting lady biologist from Italy) there was a certain diffidence about him and a sensitivity which he kept well concealed from professional colleagues. It was the presence of Jean Medawar and the great understanding between them which provided the steel. Medawar could joke that it was the fortunate possession of an entirely happy marriage which gave him more time than certain of his colleagues to concentrate on work, but the joke was true.

It is hard — and disadvantageous with reviewers — to write a loving biography, particularly of a Nobel prizewinner who lends himself on so many counts to hero worship. But Lady Medawar succeeds brilliantly: she has written an unsentimental tribute to their relationship which is also genuinely revealing of the man — a man who thought that Wagner's plots were "all rot" but was transported by the music, an immensely significant footnote to his grasp of the imaginative as a source of insight, developed in his philosophical essays. Einstein played the violin, but I have the impression that, like a grounding in philosophy, this blakean receptiveness is in short supply among biologists.

There cannot be too many extant accounts of a relationship which was happy, passionate and intellectually reciprocal on quite this scale. Moreover, in view of Medawar's "very decided preference", his single-minded determination to press on regardless despite the torpedoes, and his complete lack of any expression of self-pity in the face of massive adversity, it is most fitting that we should have this account of his illness from the woman who shared it and supported him. There have been a few great scientific love stories — the Curies come to mind — but in chronicling this one, Lady Medawar adds to our knowledge of a great man and increases our admiration for a great lady. ☐

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Chaperones of the cell

R. John Ellis

Stress Proteins in Biology and Medicine. Edited by R. I. Morimoto, A. Tissières and C. Georgopoulos. *Cold Spring Harbor Laboratory Press: 1990. Pp.450. $97.*

ALL cells contain groups of highly conserved proteins that increase rapidly in concentration when the cells are exposed to environmental stresses, including those that cause human disease. The most studied stress is high temperature. There is much indirect evidence which suggests that stress proteins have a protective function, allowing cells both to recover from the inducing stress and to survive subsequent stronger stresses that would otherwise be lethal.

The study of stress (or heat-shock) responses at the molecular level has, until recently, concentrated on the mechanisms that cause these stress proteins to accumulate. A significant component of these mechanisms is the rapid increase in transcription of genes encoding stress proteins. Much detailed information is available about the regulatory DNA sequences and DNA-binding proteins involved, although the mechanism by which stresses are sensed to trigger transcriptional activation is not known. Even more unclear is the function of the diverse groups of stress proteins. The high degree of conservation of the amino-acid sequences among these groups in all organisms, together with the observation that many of the proteins are present when the organism is not subjected to stress, suggest that these proteins have functions essential to normal cellular operations but are required to a higher degree under stress conditions. In the past few years this area of stress-protein research has been stimulated by increasing evidence suggesting that many stress proteins function as molecular 'chaperones'.

Chaperones are a distinct family of proteins required in certain cellular processes such as protein synthesis and assembly, protein transport across membranes, and the functioning of oligomeric protein complexes such as those involved in DNA replication and the recycling of endocytotic vesicles. All these processes produce changes in the state of protein folding and/or oligomerization, and so all involve the transient exposure of interactive surfaces to the intracellular environment (interactive surfaces are any regions of intra- or intermolecular contact important in maintaining the functional structure). Such exposed surfaces run the risk that they may interact 'incorrectly' with one another to produce nonfunctional three-dimensional structures. The probability of incorrect interactions occurring is known to vary widely but to be increased at high protein concentrations and high temperatures.

Molecular chaperones function by recognizing and binding to such surfaces to form stable complexes in which incorrect interactions are inhibited. These complexes are then dissociated by other proteins, often using the energy of ATP hydrolysis, under circumstances where incorrect interactions are favoured. Chaperones convey no steric information for either protein folding or protein oligomerization, and neither bind to nor are components of the final functional structures — hence the term chaperone.

This concept can readily accommodate the functions of stress proteins if it is assumed that the primary effect of stress is to cause the appearance of interactive surfaces that are recognized by chaperones. For example, this model suggests that heat shock causes proteins to denature and form incorrect aggregates, whereas heat-shock proteins inhibit these processes by binding to the interactive surfaces produced by high temperature. Thus the stress response amplifies a pre-existing function which all cells require for their operation under non-stress conditions.

The recent evidence that supports this interpretation forms the more interesting part of this collection of 18 chapters covering many aspects of stress proteins in microbial and animal cells, including diseased human cells. As well as discussion of the molecular details of stress proteins, their genes and their mode of action as chaperones, there are chapters devoted to more organismal aspects such as the physiological adjustments to fluctuating thermal environments, the febrile response, immunological consequences of stress and the use of hyperthermia in cancer therapy. The quality of discussion and presentation is high, and each chapter is well-referenced — it would be hard to find a better introduction to the latest thinking in this field.

A final heretical thought — perhaps all stress proteins will turn out to be molecular chaperones (the converse is not true), in which case we should drop the terms 'stress' and 'heat shock', as these words refer to only one specialized aspect of the much broader, essential roles of these proteins. ☐

R. John Ellis is in the Department of Biological Sciences, University of Warwick, Coventry CV4 7AL, UK.

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The importance of being alpha

W. C. McGrew

The Chimpanzees of the Mahale Mountains. Sexual and Life History Strategies. Edited by Toshisada Nishida. *University of Tokyo Press: 1990. Pp. 328. £60.*

IF ASKED about their images of wild chimpanzees, Europeans and North Americans would probably agree on the importance of Jane Goodall's contribution. Her long-term study of the Kakombe community of apes in western Tanzania means that, after 30 years of research, individuals like Flo, Fifi, Goblin and so on are well known to millions of lay people as well as to academics. This is as it should be, for more than any other person she, with the help of the National Geographic Society, has defined our Western view of wild chimpanzee nature.

But another study of wild chimpanzees in western Tanzania is arguably equally important, if not more so. This is the 25-year-long project of Toshisada Nishida and his colleagues at Kasoje in the Mahale Mountains. Their research programme, which is based at Kyoto University, has received wide acclaim in Japan, but is not well-known in the West, except among primatologists. One reason is, that unlike Goodall with *In the Shadow of Man* (1971) and *The Chimpanzees of Gombe* (1986), Nishida had not produced a book in English. That long-awaited convenience has now been supplied.

Differences in structure make direct comparisons between Nishida's volume and Goodall's impossible. While the latter has always written integrated, personal accounts, Nishida has edited an anthology of 16 chapters by 10 contributors. While Goodall has always striven for comprehensiveness, Nishida *et al.* are here more selective, as the subtitle suggests.

Finally, Goodall's forte has always been a blend of primarily qualitative, and only secondarily quantitative, description that focuses on individual apes. This richness is matched in the present volume only by Itani's chapter on his safari surveys through the Mahale Mountains and their hinterlands during the past 25 years. It is engrossing, adventurous stuff, and reiterates a technique that still has much to offer.

Of the book's other chapters, two stand out. One is Nishida's opening overview of 25 years of research, and is the single most accessible source in English on the history of Japanese primatological efforts in the region. Also exceptional is the compilatory chapter on demography and reproductive profiles. This is where longitudinal study of a long-lived species comes into its own. Impressive (such as a median birth interval of as long as 72 months) and unexpected (such as bimodal annual birth-peaks) results appear on almost every page of this chapter, which is an actuarial goldmine.

I have insufficient space to do justice to the 12 'data chapters', each of which is essentially equivalent to a meaty journal article. Their topics range from the specific (social context of the pant–grunt vocalization, age differences in ant-eating) to the more general (social relations among adult males, mother–infant relationships).

Comparisons between Gombe and Mahale highlight the unique aspects of the latter: at Mahale, the main study group, M-Group, is the biggest ever recorded for the species, with more than 100 members. This has numerous implications — for example, the joint strategy of a male and female of disappearing on a temporary 'honeymoon safari', as first detailed by Caroline Tutin at Gombe, cannot occur at Mahale. There are just too many male rivals to be evaded. Consequently the possessive tactics of the alpha male assume disproportionately greater importance. He dominates the matings of females at the time of ovulation. Again and again, the importance of being 'the alpha' (highest-ranking male) comes through. The long-time holder of this position, Ntologi, deserves just as much fame in his own right as do Washoe or Lana in theirs.

My only complaint is one of frustration. Nishida has told only a fraction of the story, and whole areas of interest like tool use are largely ignored. Is it too much to ask him to give us a fully synthetic sequel? □

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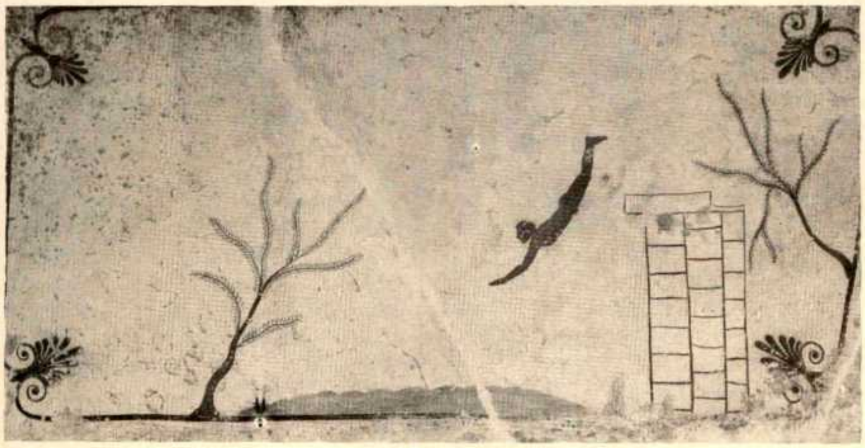


The plunge of the diver from this world to the next. The platform symbolizes the pillars of Herakles, the edge of the known world, and the water the unknown, limitless ocean beyond. The painting is on the underside of a coffin lid at Paestrum, one of the best preserved classical cities of the ancient world. From *Paestrum* by John Griffiths Pedley. Published by Thames and Hudson as part of the series "New Aspects of Antiquity", general editor Colin Renfrew. Price is £20, $35. □

Ordered growth

Lewis Wolpert

Pattern Formation. Ciliate Studies and Models. By Joseph Frankel. *Oxford University Press: 1990. Pp.314. £52, $65.*

CILIATES are bad and good for cell biologists. Bad because they undermine our self-esteem by showing how little we understand about cell structure at the molecular level; good because they offer us problems, which, when solved, will be a quantal leap in our understanding of not only cells but, surprisingly, embryos. For among the extraordinary properties of ciliates is that their mechanism of pattern formation and regulation is formally similar to that of multicellular systems.

The varied surface patterns of ciliates are highly complex and asymmetrical, yet are essentially a variation on the organization of ciliary units. Division of ciliates is a good introduction to the basic problems. When a ciliate like *Tetrahymena* divides, the process is more like budding in hydra than a typical division of a cell. In fact its growth and division is best likened to a clonal cylinder. The ciliate grows longitudinally, and before division a second set of structures develops in the posterior half. In the fission zone, in the middle of the cell, two opposite poles of the cell are thus juxtaposed. The oral apparatus defining the anterior end of the new cell begins with the formation of an anarchic field in which the ciliary units are so oriented that no order is evident. Gradually, the highly ordered organelle emerges. In addition to this local ordering there is long-range order which places the organelles in the correct relationship to each other.

Ciliates provide the best example of structural inheritance — that is, pre-existing structural organization can be inherited during their vegetative growth and division, and is not under genetic control. This is one of the very few examples of inheritance not involving an alteration in DNA. The classic example is the inheritance of ciliary rows that have been inverted by 180°. But it is not just inversion of ciliary rows that can be inherited. Large-scale reversals of asymmetry are possible so that there is a change in intracellular handedness that affects the arrangement of all the cortical structures within its domain.

Ciliates show a remarkable capacity for regeneration and pattern regulation. It is as if the cell is behaving like a classical embryonic gradient field. Frankel has proposed a formal model, based on positional information, which attempts to account for a large number of results. Treating the ciliate as a cylinder, it is assumed that there are both longitudinal and circumferential positional values giving, in effect, a two-dimensional coordinate system, which makes the different parts of the cortical system non-equivalent. One property of the system is its tendency to achieve a continuum of normally spaced positional values, and in this it is similar to the polar coordinate model that has been so successful in accounting for the regeneration of multicellular systems. A key feature of the model is that positional values are 'interpreted' by the development of specific structures. Both positional value and interpretation are, however, completely without a molecular basis. But as Frankel makes clear, the model provides a partial explanation for a large amount of information which would otherwise be incomprehensible. Moreover, there are few errors of commission, that is, a structure forming where it is not expected.

This brief review does little justice to the richness of the material, the clarity of the exposition, or how well Frankel relates ciliate patterning to that in multicellular organisms. He has written an outstanding book. One can only hope it will encourage others to enter the field. The difficulties in solving the problem are great, but so are the rewards. One cannot but believe that underlying patterning in the single cell is a fundamental biological mechanism that has been taken over by multicellular organisms. ☐

Lewis Wolpert is Professor of Anatomy, University College and Middlesex School of Medicine, Cleveland Street, London W1P 6DP, UK.

■ Two new books from Cambridge University Press also address early development. *This Side Up* by Robert Wall, describes spatial determination in the early development of animals. Price £70, $110. *Morphogenesis* by Jonathan Bard, is about the cellular and molecular processes of developmental anatomy. Price £35, $54.50. ☐

Early blooming

Ghillean T. Prance

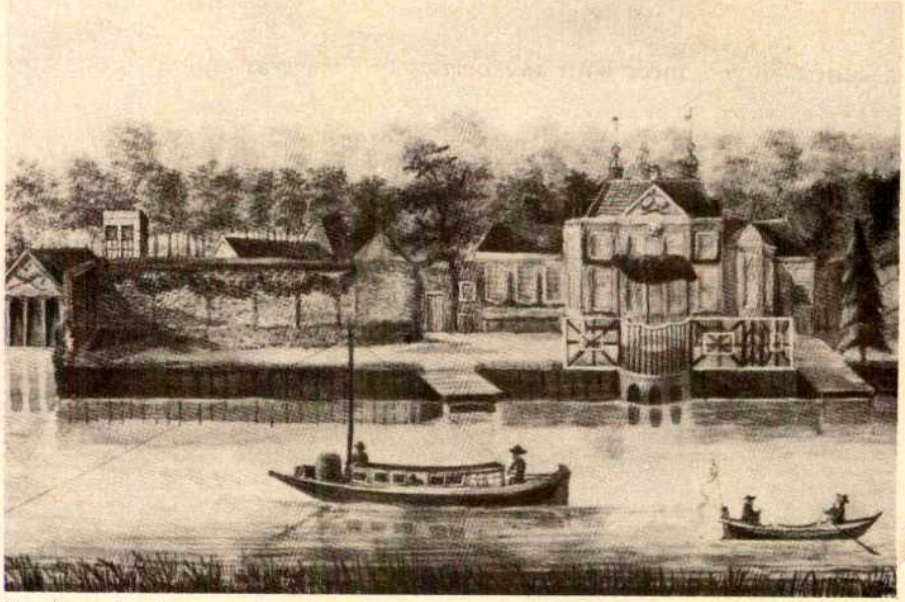
The Chelsea Gardener. Philip Miller 1691–1771. By Hazel le Rougetel. *Natural History Museum Publications, London: 1990. Pp. 212. £14.95, $29.95.*

"MILLER'S *Gardeners' Dictionary* is the most important horticultural work of the eighteenth century" says Professor William T. Stearn, who contributed the final chapter to this book. This is certainly true; indeed, Philip Miller was also the leading British gardener of his century. As the keeper of the Apothecaries' Physic Garden at Chelsea from 1722 to 1770, he built up the most richly stocked garden in Europe. Miller was both botanist and horticulturalist and his dictionary is the source of the original descriptions and names of many species of plants.

Miller was a contemporary of Linnaeus and, in the eighth and last edition of his great work, he adopted the Linnaean system of nomenclature which he had earlier resisted. He was a great correspondent and maintained contact with botanists and plant collectors from all around the world. As a result, he obtained for the Physic Garden many species from Europe, North America, South Africa and the West Indies which were not seen by Linnaeus. Miller named these both from living material and from herbarium specimens, so his dictionary is a work of major importance to taxonomic botany. Many familiar garden plants still bear the name of Miller after their Latin binomials.

This delightful new book about Miller covers many aspects of his life and his influence on the world of botany. Among his correspondents were Sir Joseph Banks and Sir Hans Sloane, and a fascinating chapter deals with his transatlantic correspondence, particularly with plant collector John Bartram. Another chapter is about Miller's most valued flowering shrub, the rose. The Provence appears to have been Miller's favourite among the many species and varieties of roses that he cultivated at Chelsea. Miller did not confine his horticulture to the garden directly under his care in Chelsea but advised and exchanged material with many of the great estates of England, such as the garden of the third Duke of Argyll at Whitton which accumulated a large collection of trees. On the death of the duke in 1761, many of the beautiful trees at Whitton were transferred by Lord Bute to Princess Augusta's garden at Kew, the precursor of the Royal Botanic Gardens. Fortunately, Miller's work at Chelsea has been continued by subsequent generations and the Physic Garden is still a testimony to its greatest keeper. This book will make any visitor's trip to today's garden even more interesting.

The book is abundantly illustrated with 16 colour plates and numerous black-and-white drawings carefully selected from Miller's *Gardeners' Dictionary* and *Figures of Plants* and engravings of Chelsea. It is attractive and well laid out, and will be of interest both to botanists and to horticulturalists who are interested in the history of their discipline. ☐



Meerburg, the house and garden of M. de la Court. Philip Miller visited this garden, at Oud-Poelgeest, Holland, in 1727, and was instructed by its gardener on the cultivation of pineapples. (From *The Chelsea Gardener*.) ☐

Ghillean T. Prance is Director of the Royal Botanic Gardens, Kew, Richmond, Surrey TW9 3AB, UK.

■ The Chelsea Physic Garden, 66 Royal Hospital Road, London SW3 4HB, UK, is open to the public on Wednesdays and Sundays from 2 p.m. to 5 p.m. ☐

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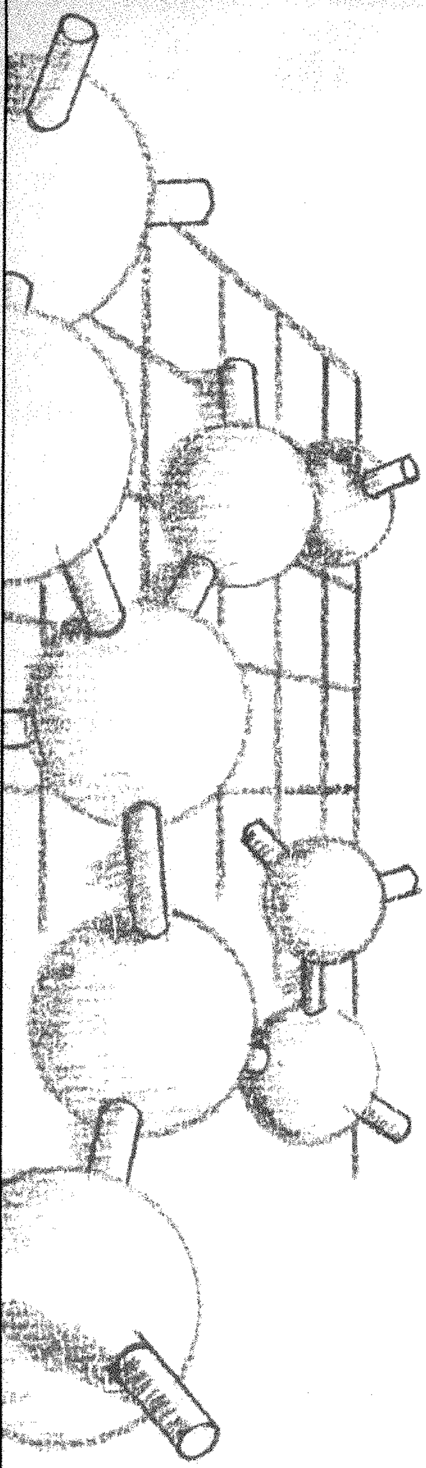

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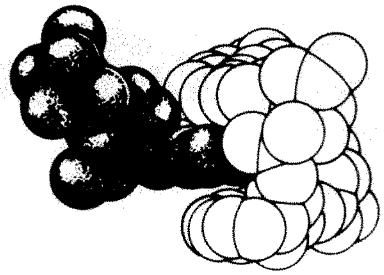

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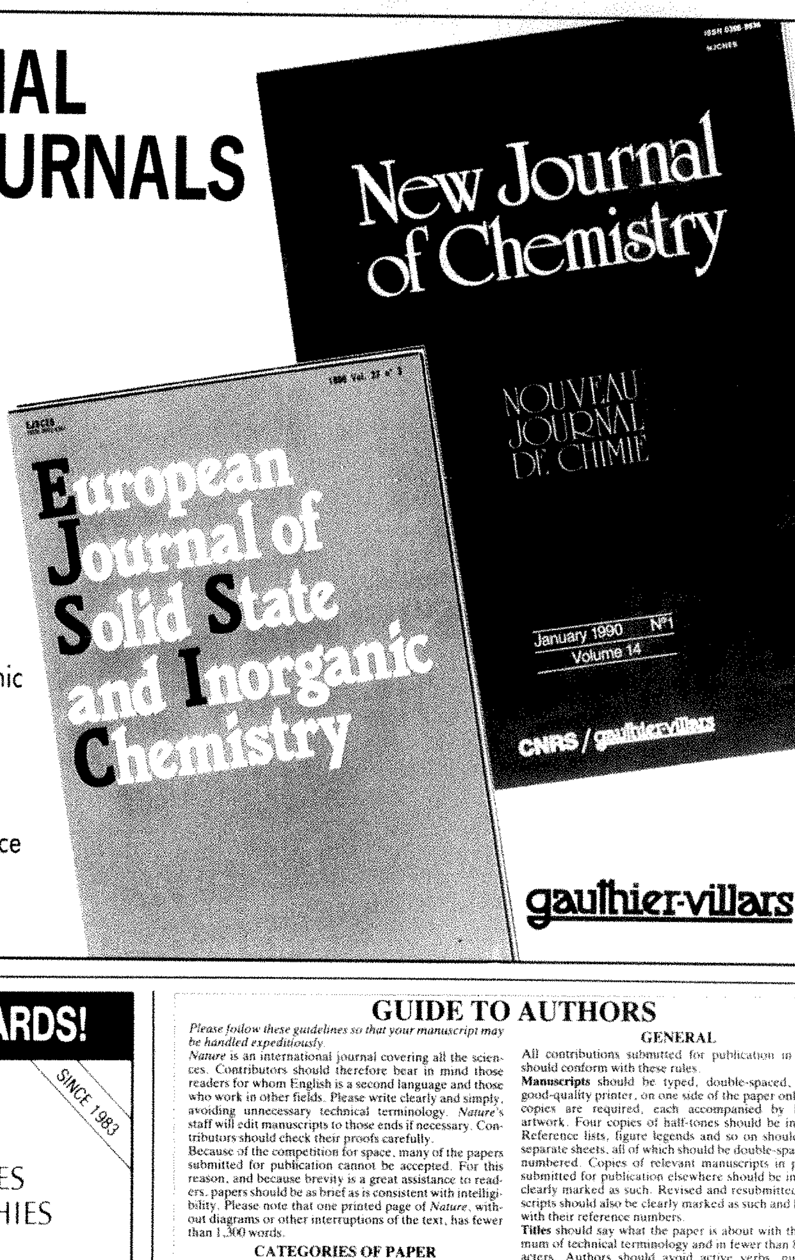

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Sun and dust versus greenhouse gases: an assessment of their relative roles in global climate change

James E. Hansen & Andrew A. Lacis

Many mechanisms, including variations in solar radiation and atmospheric aerosol concentrations, compete with anthropogenic greenhouse gases as causes of global climate change. Comparisons of available data show that solar variability will not counteract greenhouse warming and that future observations will need to be made to quantify the role of tropospheric aerosols, for example.

OBSERVATIONS of steadily increasing concentrations, principally man-made, of greenhouse gases in the Earth's atmosphere[1-3] have led to the expectation of global warming during coming decades[4-6]. Computer simulations, supported by palaeoclimate studies, suggest that the potential greenhouse climate change within a century could rival the difference between today's climate and the last great ice age of 20,000 years ago[7,8]. But the greenhouse effect is in competition with other mechanisms for climate change. Solar variability, although a speculative subject, has received much attention, and recent observational advances allow initial quantitative comparison of the effects of the Sun and the greenhouse. Another mechanism, a change in the atmospheric aerosols (natural and man-made), is of comparable importance.

Because curtailing the growth of greenhouse gases would require significant changes in global energy use and unprecedented international cooperation, it is essential to develop a good quantitative understanding of the relative importance of these different mechanisms of climate change. We also need to know how the magnitudes of such forced climate changes compare with unforced internal fluctuations of the climate system. Here we compare the above climate forcings, describe observations that would help sort out cause and effect of near-term global climate trends, and discuss implications for policy making.

Global climate can fluctuate without any change in the external forcing. For example, the Earth's simulated mean surface temperature varies as much as 0.4 °C in a 100-year run of a global climate model[9] with fixed solar irradiance and fixed greenhouse gases (Fig. 1). These fluctuations arise because the coupled nonlinear equations describing atmospheric structure and motion have solutions exhibiting chaotic behaviour. Tiny perturbations or changes of initial conditions (the flap of a butterfly's wings) give rise to solutions that are different on the timescale of months or years, and these fluctuations are magnified on decadal timescales as a result of the thermal inertia of the ocean, even if possible changes of ocean circulation are ignored[10,11]. In effect, the atmosphere and ocean do a lot of 'sloshing' around. Some of the sloshing, for example, the El Niño/Southern Oscillation phenomena[12], will eventually be predictable on a limited timescale, but most of it is of a chaotic nature for which long-term prediction is only possible in a statistical sense. An externally forced climate change must be at least comparable in magnitude to this internal noise to be clearly discernable.

A climate forcing, natural or anthropogenic, is an imposed change that modifies the planetary radiation balance, thus affecting the planetary temperature. The natural forcings that seem to be most significant, based on systematic comparison of radiative effects, are changes of stratospheric aerosols owing to large volcanic eruptions and changes of solar irradiance. The largest anthropogenic forcings seem to be increasing infrared-absorbing (greenhouse) gases, man-made tropospheric aerosols, and perhaps changes of surface reflectivity owing to desertification and deforestation.

The climate change that results from a change in the climate forcing depends on many feedback processes in the climate system. These feedbacks, including changes of clouds, water vapour, ice and snow cover, are complex and not well understood. Thus, climate sensitivity is very uncertain; for example, it is estimated that doubling the concentration of CO_2 in the atmosphere could lead to an eventual global warming anywhere in the range 1.5-5.5 °C[4,8,13,14]. Fortunately, this uncertainty can be largely avoided by contrasting the magnitude and timescale of the climate forcings rather than the resulting climate response. High-frequency changes in the forcing have less impact than a sustained forcing, because of the thermal inertia of the climate system. But uncertainties about ocean mixing rates, and thus about the effective thermal inertia of the system, only provide further reasons to focus first on climate forcing, and then discuss climate response.

Comparison of solar and greenhouse effects

A change of solar irradiance is perhaps the simplest climate forcing, and it serves as a standard for comparison. At the Earth's mean distance from the Sun, the irradiance normal to the Earth-Sun line is $\sim$1,370 W m^{-2}. Because the Earth's surface area is four times its cross-section and 30% of the sunlight is reflected back to space without being absorbed, the mean solar heating of the Earth is $\sim$240 W m^{-2}. A change of solar irradiance by 0.1% is therefore a climate forcing of $\sim$0.24 W m^{-2}.

Greenhouse and solar climate forcings of recent years can be compared accurately. Precise measurements of CO_2, the

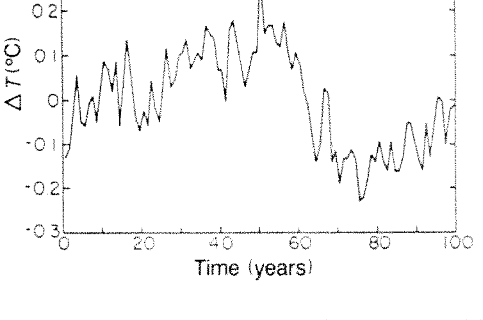

FIG. 1 Global temperature in 100-year run of a climate model[9] with no variations of climate forcing.

principal anthropogenic greenhouse gas, were initiated in 1958[1]. Since then, CO_2 has increased from 315 p.p.m. to over 350 p.p.m. The history of chlorofluorocarbons (CFCs) is also known[15], because CFCs are entirely man-made and production records are available. The trends of methane (CH_4) and nitrous oxide (N_2O), which contribute much less than CO_2 and CFCs to the greenhouse forcing over the past 30 years[15], are known approximately[16]. Ozone (O_3) , another greenhouse gas, is believed to be decreasing in the stratosphere and increasing in much of the troposphere, but the changes are so variable and poorly measured that it is impossible to say whether they cause a net global warming or cooling[15,17]. It is unlikely, however, that the ozone global climate forcing is >10-20% of the net forcing by the other gases.

The net climate forcing by CO_2, CFCs, CH_4 and N_2O for the period 1958-1989 is $>1\ W\ m^{-2}$ (Fig. 2). This is the heating of the Earth's troposphere calculated with a simple (one-dimensional radiative-convective[5]) or a more sophisticated (three-dimensional[9]) climate model for the indicated changes of these gases. We calculated an increased greenhouse forcing between 1850 and 1989 (Fig. 2) of $\sim 2\ W\ m^{-2}$. Others[16,18] have reported anthropogenic greenhouse forcing as large as $2.5\ W\ m^{-2}$, the difference being due mainly to small increases of CO_2 and CH_4 before 1850, stratospheric H_2O (which chemical models suggest may have increased because of added CH_4) and uncertain O_3 changes. We have omitted any forcing owing to O_3 or stratospheric H_2O changes because even the sign of the O_3 forcing is uncertain and there are no confirming observations of long-term stratospheric H_2O changes. The $2\text{-}2.5\ W\ m^{-2}$ greenhouse forcing is uncertain by a further 10-20% owing to imprecisions in the radiative parameters[6,19]. Our calculated history for the greenhouse climate forcing since 1958 is shown as the solid curve in Fig. 3.

Precise measurements of solar irradiance, obtained above the interfering effects of the Earth's atmosphere, were initiated in the late 1970s. A cavity radiometer[20] on the Nimbus 7 spacecraft has obtained data from late 1978 to the present, and an active-cavity radiometer[21] on the Solar Maximum satellite with more sophisticated calibrations obtained data from late 1979 through mid-1989. These instruments are believed to be capable of precisions (relative accuracies) of better than 0.1%. Despite some differences in results of the two instruments, they are consistent in showing a decline of solar irradiance of ~0.1% between 1979 and the mid 1980s, with a partial recovery of the irradiance by 1989.

Solar forcing of the climate system based on the present official Nimbus 7 data[20] is shown in Fig. 3, with zero forcing defined as the mean for the period of measurement. The combined greenhouse and solar forcing is also shown. Over the common period of accurate solar and greenhouse data the changing Sun significantly modulated the net climate forcing, but it did not alter the trend.

We stress that climate response to a given forcing depends on the history of that forcing over a period comparable to the response time of the climate system[8,10]. The response time is of the order of decades, and may be a century or more if climate sensitivity is high[8,22-24]. Because of this damping, the climate impact of a fluctuating forcing, such as the solar change in Fig. 3, is much less than that of a steady forcing. But solar irradiance may have long-term, as well as short-term, variations.

We also note that the climate system would not be expected to respond in exactly the same way to a change of solar forcing as it would to a change of greenhouse forcing of the same magnitude and timing. Solar heating, for example, is concentrated toward low latitudes, whereas greenhouse heating is more uniformly distributed; also, because solar variability is greatest at ultraviolet wavelengths[25], a substantial fraction of the change of absorbed solar energy occurs high in the atmosphere where it is less effective in warming the surface. Tests with global climate models[8,26] do, however, yield remarkably similar responses for doubled atmospheric CO_2 and for a (spectrally uniform) 2% increase of solar irradiance, both forcings being in the range $4\text{-}4.8\ W\ m^{-2}$. Thus a comparison of solar and greenhouse forcings, as in Fig. 3, seems to be meaningful to first order.

The changes of solar irradiance in the past decade are associated with changes in the area of sunspots, which are dark cool regions of reduced irradiance, and faculae, which are bright regions of increased irradiance[27,28]. The faculae effect dominates, and thus the irradiance increases in periods of maximum solar activity. If the empirical relationships between the areas of sunspots and faculae and the solar irradiance deduced from the data of past decade were valid on all timescales, longer-term changes of solar irradiance would hardly exceed 0.1%. Nor are large fluctuations of energy flow from the solar interior expected: even if the rate of nuclear energy production varied, the long diffusion time for a photon to reach the solar surface, $\sim 10^4$ years, would smooth out the variations[29]. But changes in the efficiency of convection in the outer convective layers of the Sun, caused, for example, by fluctuations in magnetic field strengths, can alter the rates of energy storage and release. Given the magnitude of surface-layer energy reservoirs, this implies that there may be important changes in the solar irradiance on timescales from decades to centuries[29].

Indeed, Eddy[30] has argued that reduced solar irradiance during the Maunder minimum of solar activity (1640-1720) may have been responsible for the Little Ice Age when global temperature is estimated to have been as much as 1 °C colder than today[31]. Other possible causes for that climate change exist, including fluctuations of ocean heat transport and the increase

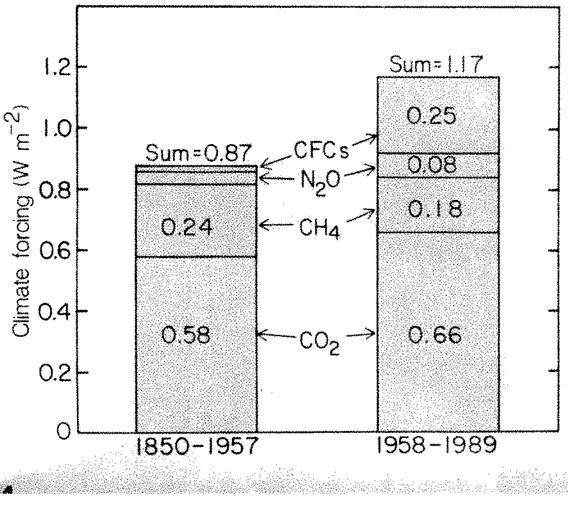

FIG. 2 Added greenhouse climate forcings ($W\ m^{-2}$) for the periods 1850-1957 and 1958-1989. Assumed gas abundances for the three times (1850, 1958, 1989) dividing the two intervals are: CO_2 (285, 315, 351 p.p.m.), CH_4 (0.8, 1.29, 1.71 p.p.m.), and N_2O (280, 289, 309 p.p.b.). Abundances for the many CFCs and references for data sources are given in ref. 15. Calculated N_2O and CFC forcings for the interval 1850-1957 are ~0.035 and 0.015 $W\ m^{-2}$, respectively.

of greenhouse gases between the 1700s and 1800s, but solar variability is one of the plausible mechanisms. Is it likely that a future decrease in solar activity may cancel greenhouse warming? That tack is taken in a recent report[32] to the chief of staff of US President Bush, which contends that solar irradiance can be expected to decline early in the twenty-first century. Thus the authors argued against measures to slow down the increase in greenhouse forcing because such efforts "could turn out to be unnecessary or even harmful if a substantial natural cooling occurs in the twenty-first century".

In comparing possible solar and greenhouse climate changes, one must take care that consistent assumptions are made about climate sensitivity. This can be done most simply by comparing the climate forcings, a simple comparison being valid because the timescales of supposed Little Ice Age forcing and recent greenhouse forcing are similar. Anthropogenic greenhouse forcing has already reached 2-2.5 W m^{-2}, equivalent to an increase of solar irradiance by ~1%. Given the increase of greenhouse forcing in the past three decades (Fig. 2), it is apparent that it will reach a level equivalent to a solar change of at least 2% by the middle of next century, unless the rate of growth of greenhouse-gas emissions is reduced.

The possibility of a decline of solar irradiance by 2%, although 20 times larger than the changes measured in the past 11 years, has not been ruled out categorically. The irradiance of some solar-type stars has been observed to change by several tenths of a per cent[33]. The Smithsonian Observatory monitored the Sun from mountain tops for the period 1902-1962, initially reporting variations of >1%. But comprehensive reviews of these data[34] concluded that the variations were due primarily to fluctuations of atmospheric transparency, and that they established only an upper limit of ~0.3% for solar variations. Evidence of solar change over a longer period is provided by measurements of the solar diameter. The diameter of the Sun is affected by thermal energy storage in its outer layers because it must maintain global hydrostatic equilibrium on timescales greater than an hour. The small changes of solar diameter measured over the past 275 years are near the limit of detectability[35]. Although the exact relation between the solar diameter and luminosity is uncertain, there probably were secular luminosity variations during that period, but they did not exceed several tenths of a per cent[31,36,37].

These results do not imply that solar variability has been unimportant in past climate change or that the Sun's effects will be negligible in the future. For example, it has been shown that an increase of solar irradiance by only ~0.3-0.4%, just within the range of possibility, is one conceivable explanation of the observed global warmth of the 1930 and 1940s[5]. Also, Wigley and Kelly[31] have shown that solar variations of several tenths of a per cent may explain many of the climate variations of the past 10,000 years.

Even if there are changes in the solar irradiance in coming decades, it is far from certain whether it will increase or decrease. One commonly used indirect measure of solar activity covering thousands of years is the record of the carbon isotope ^{14}C preserved in tree rings. ^{14}C is continuously formed in the atmosphere by incoming galactic cosmic rays, which are modulated by solar activity. Spectral analyses of the ^{14}C data, when extended into the future, suggest that solar activity will be increasing during the next several decades[38,39]. Inferences from such proxy records are, however, very uncertain. It is therefore important to compare the irradiance during the upcoming 11-year solar cycle to that of the previous cycle. This will provide the first reliable evidence of the extent to which solar change may add to, or subtract from, greenhouse forcing during coming decades.

We can conclude only that solar irradiance would need to decline by ~2% to counter greenhouse climate forcing by all the anthropogenic gases that will have accumlated in the atmosphere by the middle of next century, assuming no reductions in greenhouse emisssions. Although such a solar decline is not impossible, it is much larger than existing indications of solar variability. Furthermore, solar fluctuations can be positive as well as negative. Measurements of solar irradiance during the next decade or two are crucial for detection of any long-term trends and to help sort out cause and effect of observed climate changes.

Aerosols

Other than greenhouse gases, the largest known global climate forcing is that due to changing atmospheric aerosols. We first discuss stratospheric aerosols, which have a high degree of natural variability and have been well measured, and then tropospheric aerosols.

Stratospheric aerosols arise mainly from volcanic eruptions. Benjamin Franklin[40] noted that volcanic aerosols reflect sunlight to space, and therefore argued that the eruption of a large volcano on Iceland may have been responsible for unusual cold in 1783-4. Many subsequent studies have found a tendency for eruptions producing a large amount of aerosols over much of the Earth, such as Tambora (1815), Krakatoa (1883) and Agung (1963), to be associated with global cooling of a few tenths of a degree Celsius for a year or two after the eruption. But, because this is only comparable to unforced global temperature fluctuations (Fig. 1), cooling cannot be identified for every large volcano.

In situ stratospheric measurements during the past few decades show that the dominant volcanic aerosols are small droplets of sulphuric acid, which form from volcanic sulphur dioxide and persist a year or more after the eruption. Assuming that the aerosols of earlier volcanoes were also sulphuric acid, estimates of atmospheric transparency can be used to calculate the volcanic aerosol climate forcing[41,42,43]. The uncertainty in the resulting decadal-mean aerosol forcing is probably less that a factor of two.

The calculated volcanic climate forcing (Fig. 4) at times rivals or exceeds the greenhouse forcing, but the latter clearly dominates the long-term trend. Moreover, volcanic forcing is irregular. As discussed above, brief forcings have much less of an impact than those maintained for several decades. Thus a clustering of several volcanoes is required to have a significant impact on long-term climate. Nevertheless, it is obvious that stratospheric aerosols must be monitored to determine cause and effect of climate change during the next few decades.

Tropospheric aerosols are also an important climate forcing. In the 1970s it was speculated that increasing concentrations of anthropogenic aerosols might send the Earth into an ice age[45,46]. This speculation was probably fuelled by the fact that the Northern Hemisphere had cooled between 1940 and 1970, as well as by anecdotal information on increases of aerosols in many places. Industrialization, urban pollution, mechanized agriculture, population pressure in semiarid lands—the 'human volcano'—added noticeably to tropospheric aerosol loading. Although in certain circumstances, such as the absorbing haze in the Arctic[47,48], anthropogenic aerosols can have a warming effect, the overall direct radiative impact of man-made aerosols is clearly one of cooling[49].

In the past decade, since it was realized that global temperature was rising[5,50,51], tropospheric aerosols have received less attention than the greenhouse effect, but they have not been disproved as an important agent of climate change. Tropospheric aerosols have a global average optical depth $\tau \approx 0.1$ (ref. 52), where τ is defined by the vertical transmission of sunlight, $T = e^{-\tau}$. A 10% increase of τ yields a climate forcing of 0.2-0.3 W m^{-2}, (refs 49, 53), and the effect is nearly linear for feasible changes of τ. Although adequate measurements are unavailable, we estimate that anthropogenic aerosols comprise ~25% of all aerosols, on a global average, implying a climate forcing of 0.5-0.75 W m^{-2}. T. Anderson and R. Charlson (personal communication) argue that as much as 50% of global aerosols may be anthropogenic, implying a forcing of 1-1.5 W m^{-2}. In either case aerosol forcing is significant, but smaller than the greenhouse forcing of 2-2.5 W m^{-2}. We have argued[5] that aerosols are not a dominant global climate forcing because there is no evidence for an aerosol trend at remote locations such as Hawaii. Another argument[54] is that, despite anthropogenic aerosols being concentrated mainly in the Northern Hemisphere, temperature trends are similar in the two hemispheres, at least over the full century[50,51]. Nevertheless, the direct radiative effect of anthropogenic aerosols must counter greenhouse warming to some degree.

Because of their role as cloud condensation nuclei, a change of aerosols in the troposphere can alter the occurrence and optical properties of clouds. This aerosol effect is complicated, depending on factors such as the supersaturation spectrum of the aerosols[55], which describes the critical supersaturation at which each aerosol is activated as a condensation centre. It has been found, however, that in most situations added aerosols increase the number of active condensation nuclei and thus increase the cloud particle number density[56]. Twomey[55] therefore predicted that anthropogenic aerosols would decrease the mean cloud particle size and inhibit rainfall, and that both effects would increase cloud reflectivity.

Although evidence for such phenomena has existed for decades[57,58], dramatic reconfirmation of Twomey's expectations was obtained recently from satellite and aircraft measurements[59-61], which revealed cases of increased cloud reflectivity in trails behind ships, apparently owing to smokestack aerosols. The phenomenon was identified in shallow stratocumulus clouds under stable meteorological conditions, and was shown to involve decreased particle size[60] and decreased rainfall[61]. Qualitatively, the increased cloud reflectivity must cause a cooling, and order-of-magnitude estimates suggest that the global impact could be significant[62]. It is difficult to quantify the global significance of this climate forcing, however, because it has only been measured for extended stagnant conditions and global aerosol data are not available.

Anthropogenic aerosol/cloud climate forcing must also occur over continents, even though natural aerosol amounts are larger there. Cases of decreased cloud reflectivity have been observed in polluted industrial regions[63], but such cases seem to be the exception, not the rule. Large absorbing aerosols (such as carbonaceous ones) are common close to some sources, but the most extensive long-lived anthropogenic aerosols seem to be highly reflective sulphates formed from SO_2. Thus, in regions such as the continental United States, which has an extensive network of SO_2 sources, we can speculate about the possibility of significant aerosol or aerosol-cloud cooling. There is evidence for increased clouds over the United States[64,65], and the warming of 0.2-0.3 °C there in the past century[66,67] is less than the global warming of 0.5-0.6 °C[50,51]. This is weak circumstantial evidence, however, because the deviation of the US temperature trend from the global trend is in the range of natural variability for an area covering only 1.5% of the Earth.

We conclude that the lack of global aerosol data makes it impossible at present to determine the net anthropogenic aerosol forcing of the climate system. Observation of similar Northern and Southern Hemisphere warmings during the past century[50,51], although most anthropogenic aerosols were added in the Northern Hemisphere, is consistent with the expectation that greenhouse forcing dominates over aerosol forcing. But, as Wigley[68] has emphasized, there is sufficient observational and statistical uncertainty in the hemispheric temperature trends to mask a substantial aerosol forcing, as much as about half of the greenhouse forcing. Indeed, Durkee[69] has found evidence in satellite data of interhemispheric differences of aerosol and cloud properties, consistent with larger aerosol and aerosol-cloud effects in the Northern Hemisphere. Despite hemispheric dissimilarities in land cover and natural aerosols, such satellite data should eventually help to quantify the effect of aerosols.

Satisfactory quantitative analysis of the net climate forcing owing to anthropogenic aerosols will be difficult, because of the inhomogeneous distribution of the aerosols. It will be necessary to monitor global tropospheric aerosol properties, and carry out *in situ* case studies under a broad variety of conditions. Such data could yield the direct aerosol climate forcing, and, in conjunction with global cloud data, it could also allow evaluation of aerosol-cloud interactions.

Implications

Climate forcings. Anthropogenic greenhouse gases have increased steadily during the past century, and now cause a global climate forcing of 2-2.5 W m^{-2}. More than half of this forcing has been added in the past three decades. If emissions of these gases continue at the present or increased rates, greenhouse forcing will reach a level of at least 4-5 W m^{-2} by the middle of next century.

Solar variability may also be an important climate forcing mechanism, but solar irradiance would need to decline by 2% to counteract greenhouse forcing of 4-5 W m^{-2}. There is no evidence indicating such a decrease; in fact, ground-based observations of solar irradiance and of the solar diameter suggest an upper limit of several tenths of one per cent for solar changes in the past 275 years. There remains the possibility that small solar changes trigger larger climate forcings; speculations have included the influence of changes in the flux of ultraviolet light on ozone or the effect of energetic solar particles on atmospheric ionization and thus cloud nucleation. But no evidence has been found for a significant impact of these mechanisms on global surface temperature. Given available scientific evidence, it would be foolish to base greenhouse policy on the hope that solar variability will somehow counteract greenhouse warming.

Aerosols are an important climate forcing. A clustering of large volcanic eruptions could counter greenhouse forcing for years or even decades. But at present we cannot forecast overall volcanic activity and have no basis for anticipating a systematic long-term impact of volcanoes on net climate forcing. On the other hand, anthropogenic tropospheric aerosols constitute a significant climate forcing, due to both their direct radiative effect and their influence on cloud optical properties. Clearly, the tropospheric aerosol forcing is one of cooling, but its magnitude is very uncertain. In the absence of appropriate measurements, it is difficult to quantify the extent to which it counteracts greenhouse forcing.

Are there other important global climate forcings? By considering the Earth as a planet, specifically those factors that influence absorption of solar energy and emission of thermal

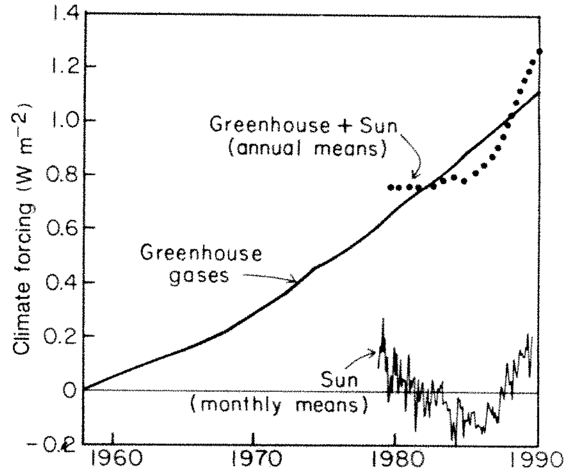

FIG. 3 Climate forcings in past three decades owing to measured changes of greenhouse gases and solar irradiance. Solar irradiance, illustrated for Nimbus 7 data[20], has been accurately measured only since 1978. Zero point of solar forcing is the 1978-1989 mean.

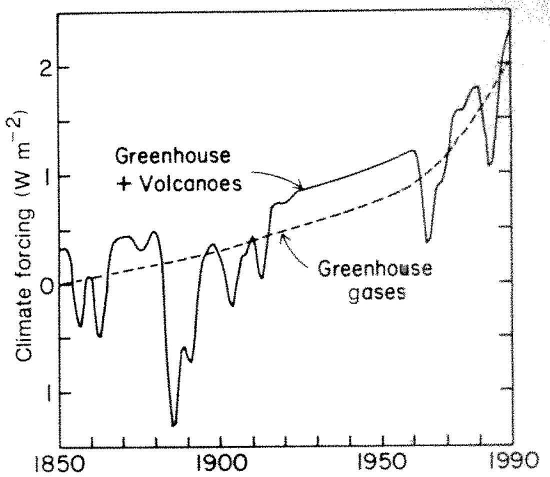

FIG. 4 Climate forcings in the past century owing to changes of greenhouse gases and stratospheric aerosols. Aerosol forcing after 1883 is based on atmospheric transmission measurements; for 1850-1883 the three largest volcanoes identified by Lamb[44] are included, with optical depth scaled relative to Agung, in proportion to the volume of ejecta. Aerosol optical depth for 1883-1960[41] is based on atmospheric transmission measurements at astronomical observatories; subsequently it is based on the transmission of sunlight through the stratosphere as recorded by approximately annual lunar eclipses[42,43]. Zero point of aerosol forcing is the 1850-1989 mean.

energy, it becomes apparent that one remaining possibility is change of reflectivity of the Earth's surface. Both deforestation and desertification tend to increase planetary reflectivity, but quantitative studies suggest that the climate impacts are confined mainly to the regions of surface change[70].

Climate response. We have so far restricted our comparisons to climate forcings thus avoiding the uncertainty about global climate sensitivity and the effect of response time. But substantial climate change must be anticipated for the forcings identified. Climate models[4-6] suggest an equilibrium global warming of 1.5-5.5 °C for doubled CO_2, which is a forcing of 4-4.5 W m^{-2}. The lower end of this range represents little net feedback, that is, it is approximately the blackbody temperature increase required to yield a flux of ~4 W m^{-2}. The upper end represents a net positive feedback of about a factor of four. This large range arises because, although feedbacks such as water vapour, sea ice and snow cover are qualitatively understood, even the direction of others such as cloud[71] and aerosol-cloud feedbacks[72], are uncertain. Recent climate model studies with alternative cloud formulations[73] reemphasize the importance of cloud feedbacks, but do not yield a climate sensitivity outside the above range. A warming of even 1.5 °C would make the Earth hotter than it has been in hundreds of thousands of years.

Lindzen[74,75] has argued that climate sensitivity may be much less than 1.5 °C for doubled CO_2. His argument, buttressed by a metaphysical presumption of a need of the planet for stability, is based primarily on the hypothesis that the net impact of increased moist convection (expected to accompany greenhouse heating) is a drying of the atmosphere, resulting in a negative water-vapour feedback. His descriptive model of moist convection includes the effect of subsidence of surrounding air, but excludes the effects of moisture detrainment at the cloud tops, cirrus anvils, large-scale dynamics and other processes. Lindzen's conclusion can be disproved on several grounds. For example, his proposed moist-convection mechanism can be tested by comparing the real atmosphere in winter and summer: at all latitudes the large-scale impact of increased summer convection is to moisten, not dry, the upper troposphere[76]. Also, satellite measurements show that even the geographical variation of the greenhouse effect[77] is in close agreement with water-vapour variations and corresponding radiative calculations, and that the maximum greenhouse effect is in regions of intense moist convection. Thus Lindzen's hypothesis of a negative water-vapour feedback cannot be reconciled with real world data, a conclusion that does not depend on uncertainties in global climate model simulations.

The best empirical measure of global climate sensitivity is provided by comparing the Earth's radiation balance in glacial and interglacial conditions. Although glacial-interglacial climate swings are associated with small changes in the Earth's orbit[78], these orbital fluctuations have little direct effect on the planetary radiation balance. Instead, the glacial-interglacial temperature differences are maintained by large changes in factors such as planetary surface reflectivity and atmospheric composition. These factors may be climate feedbacks on timescales of millennia, but on timescales of a few years or decades they are climate forcings or boundary conditions. Comparison of the glacial-interglacial global temperature change with the forcing owing to the changed boundary conditions thus provides a measure of climate sensitivity, including all fast feedback processes[8]. The last ice age, which peaked ~20,000 years ago, was 5 ± 1 °C colder than the present interglacial period, and the global climate forcing owing to changed boundary conditions (mainly changes of ice-sheet area, vegetation cover, atmospheric CO_2, CH_4 and aerosols) is estimated[8,79] to have been 7 ± 2 W m^{-2}. The inferred climate sensitivity is thus 3 °C per 4 W m^{-2} forcing. But the uncertainties in temperature and climate forcing allow a range 2-5 °C per 4 W m^{-2} forcing, a result remarkably similar to the range 1.5-5.5 °C estimated on strictly theoretical grounds. This empirical result includes not only water vapour, cloud, snow and sea-ice feedbacks, but any other feedbacks that exist in the real world, such as the aerosol-cloud feedback suggested by Shaw[80] and Charlson *et al.*[72].

One consequence of the uncertainty in climate sensitivity is an even larger uncertainty in the response time of the climate system. If climate sensitity is 1.5-2 °C for a forcing of 4 W m^{-2}, most of the warming is realized within one to two decades after the forcing is applied; but if the sensitivity is 4-5 °C, the response time may be more than a century[8,22,23]. The climate response time increases strongly with increasing climate sensitivity, more rapidly than linearly[22], because the positive climate feedbacks associated with a high sensitivity only come into play in response to the warming. Because most of the greenhouse forcing has been added very recently (Figs 2, 4), it is clear that a large part of the eventual greenhouse warming owing to anthropogenic gases already in the atmosphere has not yet occurred. This unrealized warming calls into question a 'wait and see' policy towards the greenhouse issue, because the magnitude of this climate time-bomb will grow if greenhouse-gas emissions continue to increase.

Measurements. The interest in global climate change raises the possibility of support for the measurements of climate forcings, feedbacks and diagnostic parameters, which will be needed to sort out cause and effect of climate change. The above discussion emphasizes the need for data on all significant competing factors, including solar irradiance and atmospheric aerosols.

The most precise measurements of solar irradiance recently ended as the Solar Maximum Mission was brought down by atmospheric drag; plans for the Space Shuttle to boost the Solar Maximum Mission to higher orbit were cancelled in the wake of the Challenger disaster and the resulting reduction in mission frequency. Hopefully, although the measurements are less precise, solar instruments on Nimbus 7 and Earth Radiation Budget satellites will continue to function until the planned 1992 launch of the Upper Atmospheric Research Satellite, with its active-cavity radiometer.

The difficulty of achieving adequate precision in solar-irradiance measurements is highlighted by the changes in the Nimbus 7 data that occur when corrections are made for small errors in the assumed instrument pointing[11]. The precision needed to reveal decadal trends in the solar irradiance can be provided by instruments whose sensors are internally monitored for degradation of sensitivity, as was the case for the instrument of the Solar Maximum Mission, provided there is temporal overlap of successive instruments. Because the time of failure of an instrument or satellite is not generally predictable, and because the instruments are not extremely expensive, the approach should be to have two solar instruments simultaneously in orbit, thus providing a valuable cross-check as well as continuity of calibration.

Measurements of changes of the solar diameter with the accuracy achievable from space are also important. In conjunction with the irradiance data, these will serve to clarify the relation of diameter and luminosity, and perhaps aid eventual predicatability of long-term solar changes.

Aerosols are the source of our greatest uncertainty about climate forcing. Tropospheric aerosols are difficult to monitor because of their spatial inhomogeneity, but they are a crucial variable because of the strong anthropogenic influence on their amount. Not only will it be necessary to monitor the aerosols, but also to have continued global cloud observations, because of possible interactions between aerosols and clouds.

The concept of a 'mission to planet Earth', involving satellites from the United States, Europe and Japan, is presently undergoing intense study and early development. Although intended to cover many aspects of global change, this project has potential for important contributions to the understanding of climate change, provided care is taken that it does not displace resources from other high-priority climate research and monitoring. In planning these activities it is important to recognize that a crucial requirement for climate data sets is long-term continuity. Related to this is the fact that many data sets are best obtained from a variety of conventional sources: operational weather satellites, ships, the world weather network, ground-based and aircraft special studies, and low-cost small-satellite missions. A new initiative could provide a great service by providing resources for analysis of presently available data, and by identifying and filling key gaps in the data.

Policy. In view of the enormous economic and social implications of climate change, researchers must communicate with policy makers, and, indeed, some useful advice can now be given. Based on the complexity of the scientific issues about climate forcings (such as tropospheric aerosols), climate feedbacks (such as clouds) and climate response time (such as ocean mixing and heat storage) it is clear that, contrary to recent advice delivered to the US administration[32], the scientific issues will not be settled in 3-5 years. Also, bigger computers, by themselves, will contribute little to our understanding of these problems. What is needed most is support for research using existing data, continuation and improvement of observations, and, perhaps most of all, training of people to define and analyse future data.

Nor can researchers presently provide a prescription for how the world could achieve climate stabilization, should policy-makers decide that such a goal is desirable. This is illustrated by a *gedanken* (thought) experiment concerning the rate of the use of fossil fuel. The burning of fossil fuel releases CO_2, causing about half of the anthropogenic greenhouse effect, but it also releases SO_2, forming atmospheric aerosols which partially counter greenhouse warming by reflecting sunlight and increasing cloud cover. The lifetime of added CO_2 is of the order of 10^2 years (although it cycles through the biosphere more rapidly) whereas the lifetime of sulphate aerosols is only a few days. This difference in lifetimes has important implications for the effectiveness of changes in fossil-fuel use.

First, as an extreme labelled Case I, we assume that tropospheric aerosols generated by fossil fuels have cancelled a large fraction of the anthropogenic greenhouse effect, say about half, which is approximately the amount of the greenhouse effect owing to CO_2. Case I is conceivable, because observed warming could have been caused by the other greenhouse gases. In Case I, if fossil-fuel use were stabilized (or reduced) warming would accelerate, because the short-lived anthropogenic aerosols would stabilize (or decrease) but CO_2 would continue to increase. CO_2-induced warming is eliminated in Case I only by continued exponential increase of fossil-fuel use. But that would be a Faustian bargain, because fossil fuels would run out, whereupon a huge CO_2-induced warming would begin.

As Case II, we assume that aerosol cooling is negligible compared to CO_2-induced warming. In that case, any reduction in the use of fossil fuel reduces the growth of CO_2 warming in a straightforward way. The real world almost certainly lies somewhere in the continuum between Cases I and II, but we do not know where. Thus, until the research on aerosols is carried out (a difficult task), we do not even know the direction of the change of climate forcing on decadal timescales which would be caused by a modification of fossil-fuel use.

In the presence of such uncertainties, can any useful advice be given to policy makers? We believe so. It is clearly desirable to reduce the ultimate magnitude of the 'experiment' that man is carrying out on the Earth, and there are many actions that could accomplish that and which make good sense on other grounds. These include phasing out CFCs, improving energy efficiency, increasing recycling, reducing deforestation and planting trees in appropriate places. All of these are needed for other reasons, and, in the long-term, pay for themselves. It will, however, require unprecedented international cooperation to achieve a turnaround of global greenhouse-gas emissions. The Montreal Protocol, motivated by a common desire to protect the stratospheric ozone layer, promises significant progress on CFC reductions. The first steps of an analogous process for climate protection are being taken by the Intergovernmental Panel on Climate Change, but it is difficult to predict its prospects for success.

Finally, we would be remiss if, in discussing the policy implications of anthropogenic climate change, we did not raise the issues of alternative energy sources and global population. It seems imperative that governments give much higher priority to research and development on energy sources that produce little or no greenhouse gases. Otherwise we risk the danger of soon finding ourselves, in the mid-American vernacular, up the proverbial creek without a paddle. Also, it is obvious that even sizable reduction of *per capita* greenhouse-gas emissions will be negated if global population continues to increase at the present rate. Thus governments must foster conditions leading to population stabilization, if we are to preserve the global climate and environment. ☐

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ARTICLES

Stimulation of $p21^{ras}$ upon T-cell activation

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External signals that control the activity of proteins encoded by the *ras* proto-oncogenes have not previously been characterized. It is now shown that stimulation of the antigen receptor of T lymphocytes causes a rapid activation of $p21^{ras}$. The mechanism seems to involve a decrease in the activity of GAP, the GTPase-activating protein, on stimulation of protein kinase C. In lymphocytes, $p21^{ras}$ may therefore be an important mediator of the action of protein kinase C.

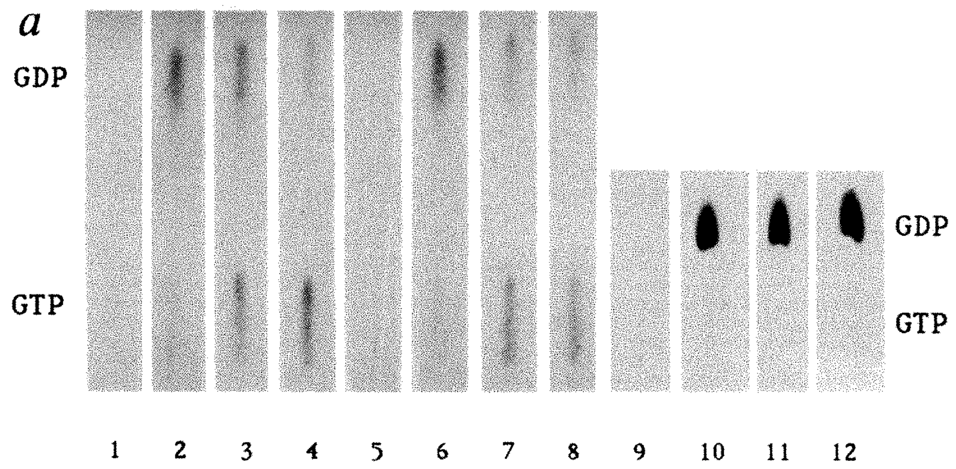
THE activated *ras* oncogenes (Ha-, Ki- and N-*ras*) can transform mammalian cells in culture and have been implicated in the formation of a high proportion of human tumours[1]. The *ras* oncogenes encode closely related proteins of relative molecular mass 21,000 ($p21^{ras}$) that bind GTP and catalyse its hydrolysis to GDP. Ras proteins can stimulate cellular growth and other events when they are bound to GTP ('active') but not when they are bound to GDP ('inactive')[2–4]. Mutations that allow $p21^{ras}$ to transform cells all cause accumulation of the GTP-bound form of the protein; many inhibit the intrinsic GTPase activity of $p21^{ras}$ (ref. 5) and others increase the rate of exchange of bound nucleotide with cytoplasmic pools, which contain much more GTP than GDP[6].

No cellular stimulus has previously been identified that controls the activation state of mammalian $p21^{ras}$, as measured by the amount of GTP bound to it relative to GDP. But a likely component of the *ras* pathway has been identified in GAP, a protein that stimulates the hydroloysis of GTP on $p21^{ras}$, thereby causing it to enter the inactive GDP-bound state[7]. Ways in which the activity of GAP might be regulated remain the subject of speculation.

Here we report that $p21^{ras}$ is indeed part of a signal transduction pathway and that in T lymphocytes its activation state can be rapidly regulated by an extracellular stimulus operating on a cell-surface receptor.

Stimulation of $p21^{ras}$

The activation state of $p21^{ras}$ was measured in intact T cells by metabolic labelling with $[^{32}P]$orthophosphate and then lysing them and immunoprecipitating $p21^{ras}$. Figure 1*a* is an autoradiogram showing the nucleotides bound to the endogenous $p21^{ras}$ in human peripheral blood lymphoblasts (PBLs)[8] and in the human T-cell line Jurkat[9]. Although $p21^{ras}$ from untreated cells is almost entirely GDP-bound, on treatment with the T-cell-activating lectin phytohaemagglutinin (PHA)[10] or CD3-specific monoclonal antibody UCHT-1 (ref. 11), a large amount of GTP accumulates on $p21^{ras}$. In the cell line HPB-ALL, where the antigen receptor is uncoupled from phosphatidyl inositol (PtdIns) turnover[12], reagents specific for the antigen receptor fail to influence the nucleotide bound to $p21^{ras}$ (Fig.



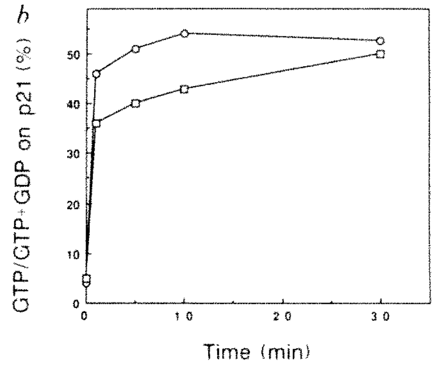


FIG. 1 Effect of T-cell activation on the nucleotide bound to p21^{ras}. *a*, Thin layer chromatogram of the nucleotides eluted from immunoprecipitates of p21^{ras} from human peripheral blood lymphoblasts labelled with ^{32}P-orthophosphate (PBLs, 1–4), J6 Jurkat cells (5–8) and HPBALL cells (9–12). Immunoprecipitation was with monoclonal antibody Y13-259 in all lanes except 1, 5 and 9, where no specific antibody was added. Cells were unstimulated (2, 6, 10) or stimulated for 30 min with 10μg ml^{-1} monoclonal antibody UCHT-1 (3, 7, 11) or PHA (1, 4, 5, 8, 9, 12). The positions of GTP and GDP standards are indicated. *b*, Time course showing the nucleotides bound to p21^{ras} on stimulation of PBLs with PHA (○) or UCHT-1 (□). The p21^{ras} was immunoprecipitated with antibody 259 from cells labelled with ^{32}P-orthophosphate that had been stimulated for the indicated times. Nucleotides were separated by TLC and quantitated by direct scanning for β radiation.

METHODS. Cells were isolated, cultured and labelled as described previously[8]. Lysis was performed in 50 mM HEPES buffer, pH 7.4, 1% Triton X-100, 100 mM NaCl, 5 mM $MgCl_2$, 1 mg ml^{-1} BSA, 10 mM benzamindine, 10 μg ml^{-1} leupeptin, 10 μg ml^{-1} aprotinin, 10 μg ml^{-1} soybean trypsin inhibitor. Nuclei were removed by centrifugation at 15,000*g* for 2 min and 0.5 M NaCl, 0.5% deoxycholate and 0.05% sodium dodecyl sulphate added to the lysate. Immunoprecipitation was for 40 min using antibody 259 precoupled to protein A-agarose by rabbit anti-rat immunoglobulin. All immunoprecipitations were in duplicate with non-specific controls. Immunoprecipitates were washed with 8 × 1 ml of 50 mM HEPES, pH 7.4, 500 mM NaCl, 5 mM $MgCl_2$, 0.1% Triton X-100, 0.005% SDS and nucleotide eluted with 2 mM EDTA, 2 mM DTT, 0.2% SDS, 0.5 mM GTP, 0.5 mM GDP at 68 °C for 20 min. Separation of eluted nucleotide was on PEI-cellulose plates run in 1.2 M ammonium formate, 0.8 M HCl.

1*a*). The increases in GTP levels on cell stimulation are rapid: they are clearly visible within one minute and are maximal by 10 minutes (Fig. 1*b*). The amount of GTP bound to p21^{ras} as a proportion of total nucleotide rises to nearly 50% after treatment with PHA or UCHT-1; in untreated cells the level is ~5%.

Protein kinase C mediates p21^{ras} activation

Stimulation of the antigen receptor in T lymphocytes is known to activate several signalling pathways[10]. To determine which of these is involved in the activation of p21^{ras}, downstream components were directly activated by pharmacological agents. Figure 2*a* shows the effects of the calcium ionophore ionomycin and the protein kinase C activator phorbol dibutyrate (PDBu) on the amount of GTP bound to p21^{ras}. The phorbol ester causes a great stimulation of p21^{ras}, but ionomycin does not affect the nucleotide bound to p21^{ras}. The fact that PDBu causes a greater stimulation of GTP levels on p21^{ras} than do the agents that act on the T-cell antigen receptor indicates that protein kinase C is likely to be a principal intracellular mediator of the activation of p21^{ras} in these cells. This is consistent with the observation that in HPB-ALL cells PDBu stimulates p21^{ras}, whereas PHA and UCHT-1, which in these cells fail to activate PtdIns turnover and hence protein kinase C[12], do not. As short-term treatment with PDBu has no measurable effects on phospholipid metabolism in T cells (J.D.G. and D.A.C., unpublished observations), we believe that the PDBu-dependent effects are the result of activation of protein kinase C and not direct lipid effects on GAP itself.

The action of PDBu is very rapid, with maximal stimulation occurring within one minute, which is faster than with antigen receptor agonists (Fig. 2*b*). This is again compatible with protein kinase C mediating the effect of antigen receptor on p21^{ras} activation. Stimulatory effects of PDBu on p21^{ras} occur at concentrations as low as 5 ng ml^{-1} (Fig. 2*c*): this is similar to the level of PDBu needed to activate protein kinase C[13]. Lysates from PDBu-treated or control ^{32}P-labelled PBLs were mixed with lysates from unlabelled cells (Fig. 2*d*); treatment of the unlabelled cells has no influence on labelled GTP bound to p21^{ras}, excluding the possibility of a post-lytic artefact.

p21^{ras} activation in permeabilized cells

Two likely mechanisms for the observed activation of p21^{ras} might involve a decrease in GTP hydrolysis on p21^{ras}, possibly due to inactivation of GAP, the GTPase activating protein[7], or an increase in the rate at which guanine nucleotide exchanged onto and off p21^{ras}. To address these possibilities, we studied the activation state of p21^{ras} in an alternative way using peripheral blood lymphoblasts that had been permeabilized with streptolysin O (ref. 14). When the permeabilized cells are exposed to [α-^{32}P]GTP, the labelled nucleotide binds rapidly to p21^{ras}, which can then be immunoprecipitated and analysed. Very little GTP accumulates on p21^{ras} from untreated cells, but significant amounts of GTP are seen on p21^{ras} from cells treated with PHA or PDBu (Fig. 3*a*). The rate at which total nucleotide binds is rapid and identical whether or not the cells have been treated with PDBu (Fig. 3*b*, *c*). Even at 27 °C (where the exchange reaction is slower than at 37 °C) PDBu treatment does not influence the binding rate. It is very striking that the exchange of nucleotide onto p21^{ras} is so rapid in permeabilized lymphoblasts; half-maximal binding is achieved in about one minute at 37 °C, whereas for pure p21^{ras} or p21^{ras} in isolated membrane preparations (either from quiescent or stimulated cells) about 60 minutes would be required to reach the same level of binding in the same buffer and at the same temperature. This indicates that an activity exists in the permeabilized cells that stimulates the exchange of nucleotide on p21^{ras}: the activity is lost or inactivated in membrane preparations. This exchange factor seems to be constitutively active for both quiescent and stimulated cells. Exchange factors for $p21^{ras}$ have recently been identified[15-17].

As shown in Fig. 3*d* (37 °C) and Fig. 3*e* (27 °C), $p21^{ras}$ is almost totally GDP-bound in the unstimulated cells, but in the cells treated with PDBu significant amounts of GTP accumulate on $p21^{ras}$. As the ratio of total labelled GTP to GDP free in the permeabilized cells is about 1:1 and is not altered by PDBu treatment (data not shown), the difference in the level of GTP bound must be due to a decrease in the hydrolysis of GTP on $p21^{ras}$ in the cells where protein kinase C has been activated.

Protein kinase C inhibits GAP activity

A possible mechanism for this inhibition of the GTPase activity of $p21^{ras}$ could be the inactivation of GAP on stimulation of protein kinase C within the cell. The activity of GAP in a Triton X-100 lysate of PBLs that had been treated for two minutes with PDBu was compared with the GAP activity in lysates from untreated cells. The ability of a lysate from PDBu-treated cells to induce hydrolysis of GTP on $p21^{ras}$ is six times less than that of a lysate from untreated cells (Fig. 4). Okadaic acid, a potent inhibitor of phosphatases 1 and 2a, was included in the incubation of the cells with or without PDBu and also in the lysis buffer; okadaic acid alone had no effect on GAP activity. As the inhibition of GAP activity by PDBu treatment is seen in whole cell lysates, it seems that the effect is on the activity of GAP but not on its localization in the cell.

Protein kinase C controls N-Ras and Ki-Ras

Protein kinase C phosphorylates $p21^{Ki\text{-}ras4B}$, although no effect of this modification on the behaviour of the Ras protein has been reported[18]. The stimulaton of GTP levels on $p21^{ras}$ as a proportion of total nucleotide is in fact higher when antibody 259, which recognizes all Ras proteins, is used than when antibody 238, which recognizes only $p21^{Ha\text{-}ras}$ and $p21^{Ki\text{-}ras}$, is used (Fig. 5*a*). Reimmunoprecipitation of the supernatants remaining after immunoprecipitation with antibody 238 shows that $p21^{N\text{-}ras}$

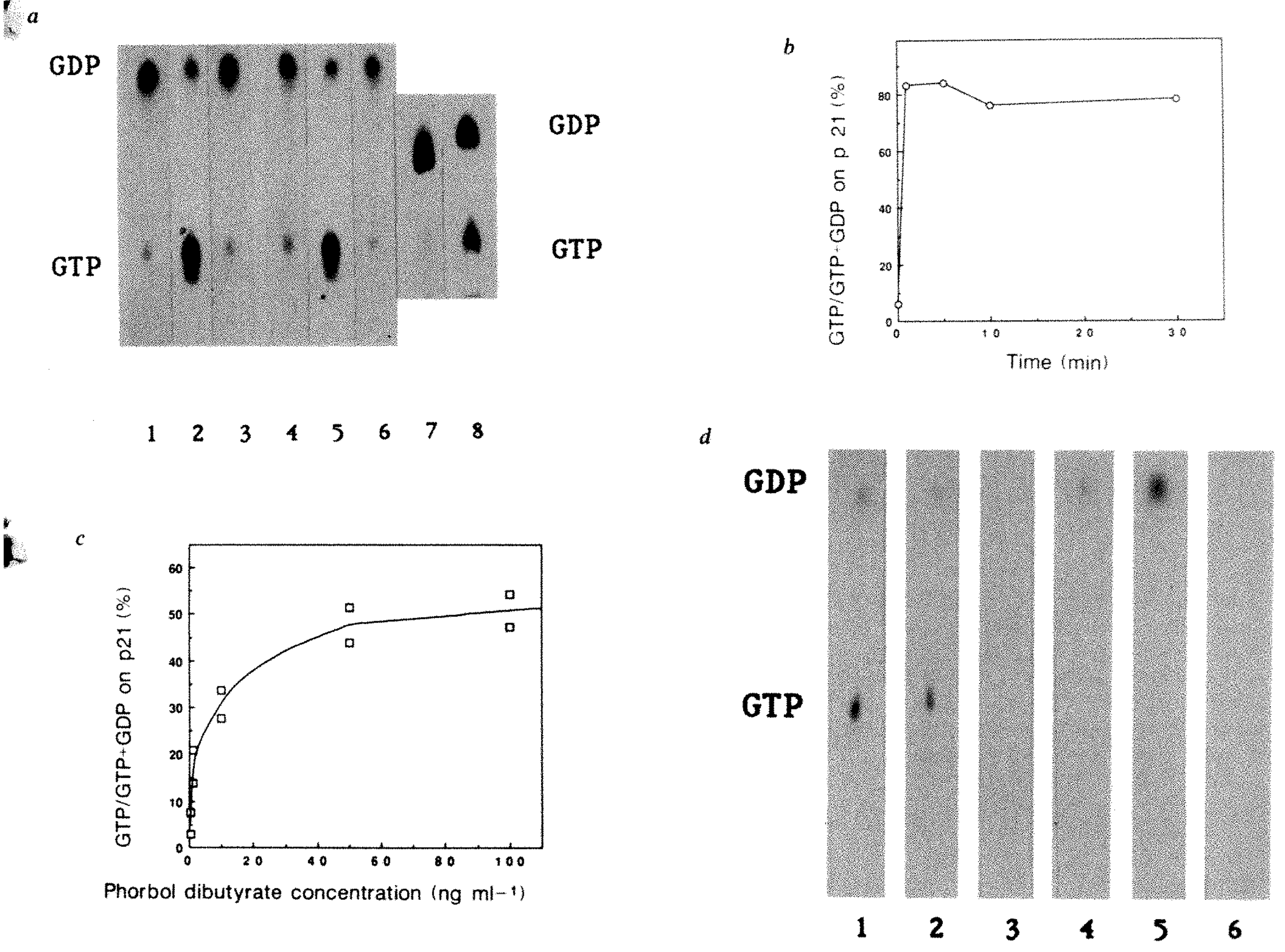


FIG. 2 Effect of phorbol ester treatment on the activation state of $p21^{ras}$. *a*, Thin layer chromatogram of the nucleotides eluted from immunoprecipitates of $p21^{ras}$ from human PBLs labelled with ^{32}P-orthophosphate (1, 2, 3), Jurkat cells (4, 5, 6) and HPB-ALL cells (7, 8). Immunoprecipitation was with monoclonal antibody Y13-259. Cells were unstimulated (1, 4, 7) or stimulated for 30 min with 100 ng ml^{-1} PDBu (2, 5, 8) or 5 μg ml^{-1} ionomycin (3, 6). The position at which GTP and GDP standards ran is indicated. *b*, Time course showing the nucleotides bound to $p21^{ras}$ on stimulation of PBLs with 100 ng ml^{-1} PDBu. The $p21^{ras}$ was immunoprecipitated with antibody 259 from cells labelled with ^{32}P-orthophosphate that had been stimulated for the indicated times. Nucleotides were separated by TLC and quantitated by direct scanning for β radiation. *c*, Phorbol dibutyrate concentration dependence of the nucleotides bound to $p21^{ras}$ in PBLs. The $p21^{ras}$ was immunoprecipitated with antibody 259 from cells labelled with ^{32}P-orthophosphate that had been stimulated for 30 min with the indicated concentration. Nucleotides were separated by TLC and quantitated by direct scanning for β radiation. *d*, Thin layer chromatogram of the nucleotides eluted from immunoprecipitates of $p21^{ras}$ from human PBLs labelled with ^{32}P-orthophosphate that had been treated with 100 ng ml^{-1} PDBu for 30 min before lysis (1, 2, 3) or were untreated (4, 5, 6). Immunoprecipitation was with antibody 259 (1, 2, 4, 5) or non-specific antibody (3, 6). Immediately after lysis the cells were mixed with lysates from identically prepared unlabelled cells that had been treated with PDBu (1, 3, 4, 6) or were untreated (2, 5).

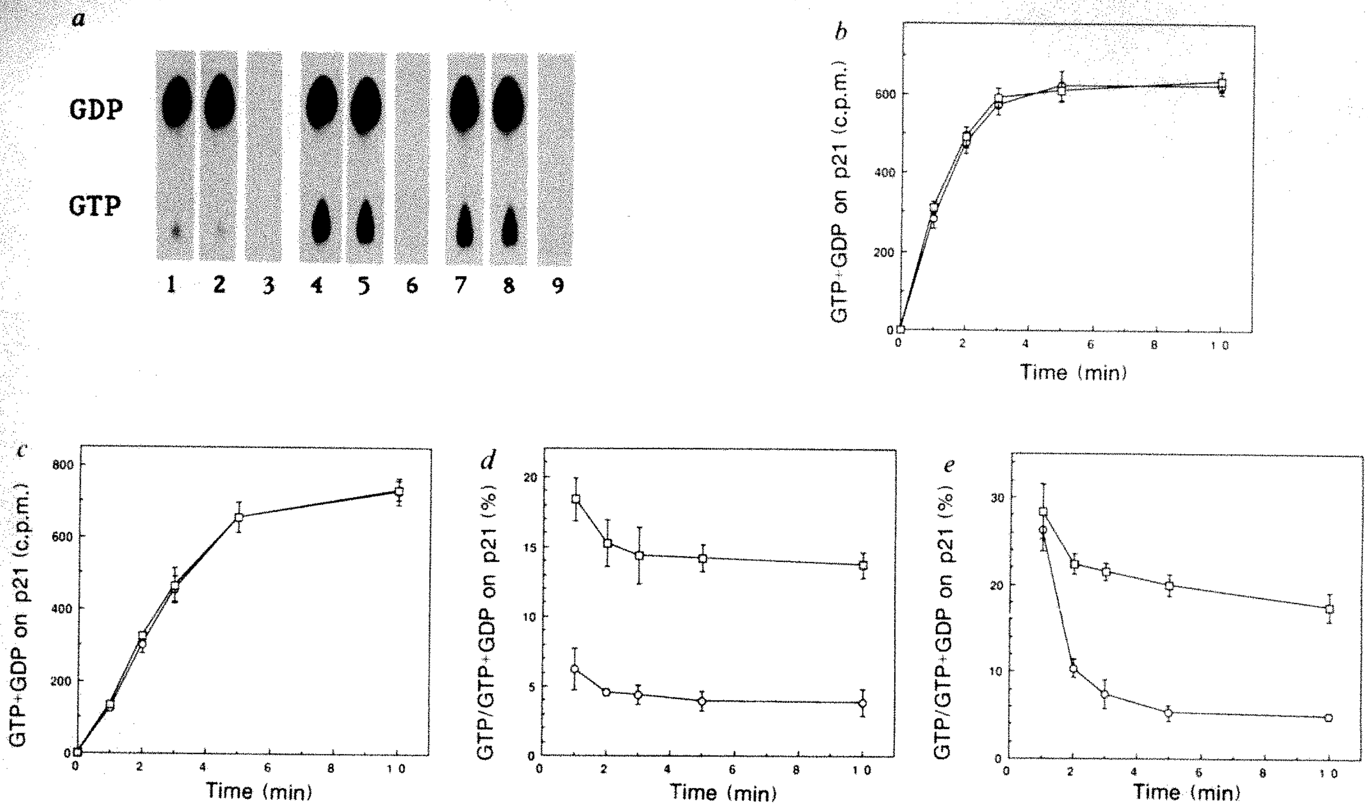


FIG. 3 Effect of antigen receptor or protein kinase C activation on nucleotide binding to p21^{ras} in permeabilized cells. *a*, Thin layer chromatogram of the nucleotides eluted from immunoprecipitates of p21^{ras} from permeabilized human PBLs that had been exposed to [α-^{32}P]GTP for 5 min. The cells were quiescent (1, 2, 3), treated with 10 μg ml^{-1} PHA (4, 5, 6) or 100 ng ml^{-1} PDBu (7, 8, 9). Immunoprecipitation was with monoclonal antibody Y13-259 in all lanes except 3, 6 and 9, where no specific antibody was added. GTP and GDP standards are indicated. *b*, Time course of total α-^{32}P-labelled nucleotide bound to p21^{ras} in permeabilized PBLs treated with 100 ng ml^{-1} PDBu (□) or quiescent (○) at 37 °C. *c*, Time course as *b* but at 27 °C. *d*, Time course of the amount of [α-^{32}P]GTP as a percentage of total α-^{32}P-labelled nucleotide bound to p21^{ras} in permeabilized PBLs treated with 100 ng ml^{-1} PDBu (□) or quiescent (○) at 37 °C. *e*, Time course as *d* but at 27 °C. The level of nucleotide binding seen is consistent with all p21^{ras} in the cells associating with labelled nucleotide.

METHODS. Human PBLs were permeabilized[14] by addition of 0.4 IU ml^{-1} of streptolysin O in 10 mM PIPES buffer, pH 7.2, containing 120 mM KCl, 30 mM NaCl, 1 mM ATP, 5 mM free Mg^{2+}, 100 nM free Ca^{2+}. [α-^{32}P]GTP (5 μCi, 3,000 Ci mmol^{-1}) was used for each assay point of 0.5 ml. Streptolysin O, [α-^{32}P]GTP and PDBu or lectin were added simultaneously. The p21^{ras} was recovered at the end of the incubation as described in Fig. 1.

alone also has a large amount of GTP bound to it after PDBu stimulation. Immunoprecipitation of p21^{ras} from lysates of [^{35}S]methionine-labelled PBLs (Fig. 5*b*) indicates that these cells contain similar quantities of p21$^{\text{N-}ras}$ and p21$^{\text{Ki-}ras\text{4B}}$, but little p21$^{\text{Ha-}ras}$ or p21$^{\text{Ki-}ras\text{4A}}$. Protein kinase C stimulation therefore has at least as great an effect on the activity of the N-*ras* protein as it does on the Ki-*ras* protein.

Discussion

The data presented show that in T cells the activation state of p21^{ras} is controlled by the antigen receptor. To our knowledge, this is the first time that a cellular stimulus has been shown to control the activation state of mammalian Ras proteins. The mechanism by which p21^{ras} is stimulated on T-cell activation seems to involve the protein kinase C inhibition of a GTPase

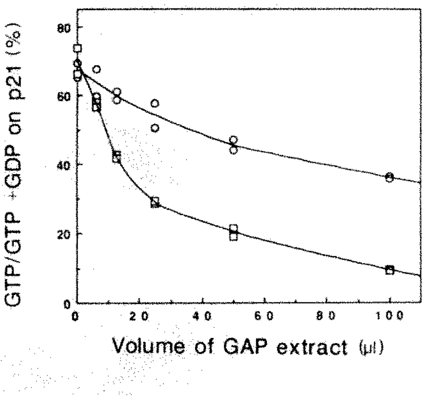


FIG. 4 GAP activity in lysates of peripheral blood lymphoblasts: effect of phorbol ester treatment. Lysates were made from 5 × 10^{8} PBLs that were either quiescent (□) or treated with 100 ng ml^{-1} PDBu (○) for 2 min and assayed for GAP activity at a range of dilutions.

METHODS. Okadaic acid (100 nM) was included in the 2-min PDBu and control incubations. Lysates were made by disrupting cells in 0.5 ml 10 mM PIPES buffer, pH 7.2, containing 120 mM KCl, 30 mM NaCl, 5 mM free Mg^{2+}, 100 nM free Ca^{2+}, 1% Triton X-100, 10% glycerol, 100 nM okadaic acid. The lysate was centrifuged at 300,000*g* for 10 min and the supernatants assayed for GAP activity. Pure bacterially expressed wild-type human p21$^{\text{c-Ha-}ras}$ (50 ng) was allowed to bind 25 μCi of [α-^{32}P]GTP in the presence of 5 mM EDTA at 37 °C for 5 min, then 10 mM MgCl$_2$, 1 mM GTP and 1 mM GDP were added and 0.75 μCi aliquots added to varying amounts of GAP extracts made up to the same volume with homogenization buffer. The mixtures were incubated for 5 min at 37 °C, the reactions stopped by addition of 10 volumes of cold lysis buffer containing 0.5 M NaCl and p21^{ras} immunoprecipitated using antibody 259. The proportion of [α-^{32}P]GTP to total labelled nucleotide on p21^{ras} was calculated following separation of GTP from GDP by TLC and quantitation by direct scanning for β-radiation.

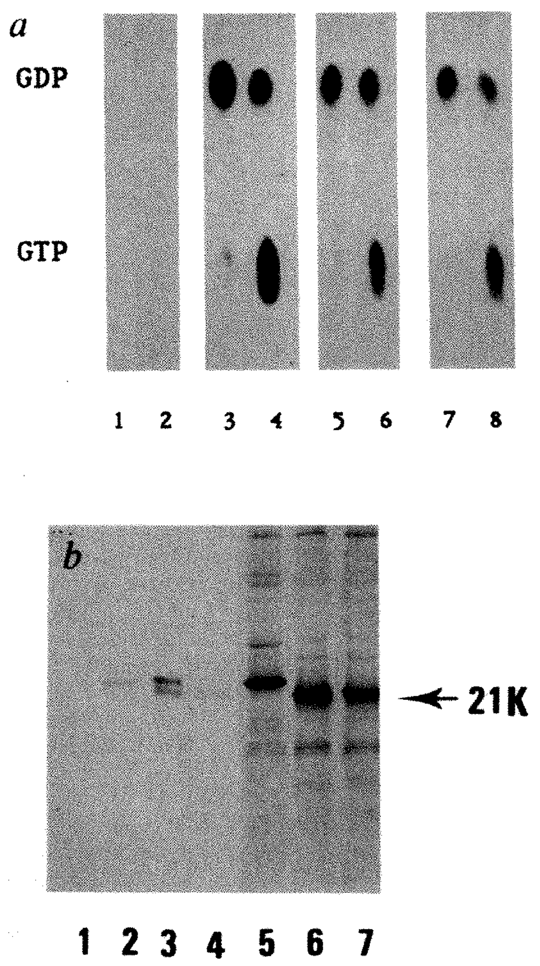


FIG. 5 Effect of protein kinase C activation on the different types of p21^{ras} in peripheral blood lymphoblasts. *a*, Thin layer chromatogram of the nucleotides eluted from immunoprecipitates of p21^{ras} from human PBLs labelled with [^{32}P]orthophosphate that were unstimulated (1, 3, 5, 7) or stimulated for 10 min with 100 ng ml^{-1} PDBu (2, 4, 6, 8). The antibodies used in the immunoprecipitations were nonspecific (1, 2), Y13-259 (3, 4), Y13-238 (5, 6) or Y13-259 on the lysates remaining after immunoprecipitation with Y13-238 (7, 8). *b*, SDS-polyacrylamide gel of immunoprecipitates of p21^{ras} from a lysate of PBLs labelled with [^{35}S]methionine. The antibodies used were non-specific (1), Y13-238 (2), Y13-259 (3), Y13-259 on the lysates remaining after immunoprecipitation with Y13-238 (4). Also shown are Y13-259 immunoprecipitations from the cell line Rat-1.K-ras25 that overexpresses human p21$^{Ki\text{-}ras4B}$ (5), the cell line Rat-1.H-ras13 that overexpresses human p21$^{Ha\text{-}ras}$ (6) and the cell line Rat-1.N-ras16 that overexpresses human p21$^{N\text{-}ras}$ (7). Cells were labelled with [^{35}S]methionine as described[21,8] and lysis and immunoprecipitation carried out as for Fig. 1. For the lymphoblasts 2×10^7 c.p.m. were used per immunoprecipitation and 5×10^5 c.p.m. for lane 6 and 2×10^6 c.p.m. for lanes 5 and 7.

activating protein, presumably GAP. The way in which activated protein kinase C brings about inactivation of GAP remains to be characterized. The simplest mechanism would be a direct phosphorylation of GAP by protein kinase C; however, we have failed to find any evidence of phosphorylation of GAP stimulated by phorbol esters in PBLs or Jurkat cells (data not shown). It is perhaps more likely that protein kinase C phosphorylates a different protein that in turn acts as an inhibitor of GAP. The mechanisms described here seem to be quite different from the previously reported tyrosine kinase phosphorylation of GAP[19,20] whose functional significance remains unclear. Alterations in the levels of tyrosine phosphorylation in response to phorbol ester treatment have not been demonstrated in lymphocytes.

The very rapid nature of the conversion of p21.GDP to p21·GTP in whole cells on activation of protein kinase C indicates that the rate of exchange of nucleotide on p21^{ras} in the cell must be very fast compared with that on isolated p21^{ras}. The data from the permeablized cell experiments confirm this and further show that the exchange rate is constitutively elevated whatever the growth state of the cells. It therefore seems that in the cell, fresh guanine nucleotide (primarily GTP, which predominates over GDP in the cytoplasm) is rapidly exchanging onto p21^{ras} because of the action of an exchange protein. In the quiescent lymphoblast the high activity of GAP ensures that bound GTP is rapidly hydrolysed and the balance of nucleotide on p21^{ras} remains in favour of GDP. On activation of protein kinase C the activity of GAP is reduced several fold, allowing the exchange protein to predominate and the nucleotide balance to swing rapidly over to GTP. Thus the activation state of p21^{ras} is the result of a dynamic balance between influences of GAP and of the exchange protein: in this system it seems that GAP is the main target for regulation, although it is clearly possible that the exchange protein could also be regulated. □

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Presynaptic mechanism for long-term potentiation in the hippocampus

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Experiments analysing the statistical properties of synaptic transmission, before and after the induction of long-term potentiation (LTP), suggest that expression of LTP largely arises in a presynaptic mechanism—an increased probability of transmitter release.

LONG-TERM potentiation (LTP) is a long-lasting use-dependent increase in the efficacy of excitatory synaptic transmission in the brain that is thought to underlie certain forms of learning and memory[1,2]. Although the trigger for LTP is generally agreed to occur in the postsynaptic cell[3-8], the site at which it is expressed is still disputed. Some authors have suggested that the mechanism of LTP is enhanced neurotransmitter release[9,10], others that it is increased postsynaptic sensitivity to transmitter[11,12], and still others that both may occur, perhaps in sequence[13]. Here we describe experiments analysing the statistical properties of synaptic transmission, before and after the induction of LTP, in both hippocampal cultures and patch-clamped slices.

Quantal analysis

Since the classic work of Del Castillo and Katz[14], the standard way to determine whether a phenomenon is pre- or postsynaptic has been to carry out a quantal analysis[15]. Such an analysis provides estimates of a, the amplitude of response to a single quantum of neurotransmitter, p, the probability of release, and N, the number of release sites. Synaptic transmission becomes more efficacious if a, N, or p is increased. In standard treatments, an increase in either N or p indicates a presynaptic mechanism, whereas a postsynaptic mechanism, such as greater receptor sensitivity, would produce an increase in a.

Quantal analysis has, however, proved difficult in the central nervous system for three main reasons. First, only rarely can one find a pair of cells that are monosynaptically connected[16]. The formalism for quantal analysis can be extended to include some situations in which more than one input to a neuron is stimulated, but such an extension is especially uncertain for classes of synapses where the Del Castillo and Katz statistical mechanism for release has not been confirmed. The second difficulty is that the characterization of the quantal event, the miniature excitatory postsynaptic current (m.e.p.s.c.), is uncertain both because spontaneous m.e.p.s.cs cannot usually be associated with a specific subset of the many synapses on a typical central neuron, and because cable filtering alters the amplitude and time course of m.e.p.s.cs that originate at a distance from the recording site[15,17]. Finally, the background noise in intracellular recording is frequently so large that m.e.p.s.cs are obscured[18,19].

We have circumvented these difficulties by studying neuronal connections in culture, where synaptically connected pairs of cells can be identified and where m.e.p.s.c. release can be evoked at defined locations so that the inherent properties of m.e.p.s.cs can be separated from cable filtering effects. M.e.p.s.cs at synapses in cultures of hippocampal neurons vary considerably in size, and the analysis of this variability has recently been extended in two ways[20]. First, m.e.p.s.c. amplitude fluctuations have been demonstrated to arise at single boutons (rather than reflecting the properties of a population of boutons); second, m.e.p.s.cs in hippocampal slices have been shown to exhibit about the same variability in size previously seen in culture[21]. An accurate quantal analysis of synaptic transmission in the hippocampus must take these properties of m.e.p.s.cs into account (see refs 22, 23).

Analysis in dissociated neurons

In a preliminary series of three experiments in culture, we used the nystatin perforated patch technique[24] to observe m.e.p.s.cs in a voltage-clamped neuron before and after briefly perfusing the bath with solution containing no Mg^{2+}. Such perfusion causes cells in the dish to fire spontaneous bursts of action potentials repeatedly and should have potentiated many synapses. Assuming LTP had been induced in a significant number of the neuron's synapses, we should have observed an increase in the mean size of m.e.p.s.cs if expression of LTP is postsynaptic. No detectable change in m.e.p.s.c. size was found up to 96 min after the induction of massive activity in the culture, suggesting that expression of LTP is not postsynaptic. The difficulty with this experiment was, of course, that we could not be sure that the synapses whose m.e.p.s.cs we recorded had undergone LTP. Therefore, we turned to a more conventional protocol.

At the outset we failed to observe LTP in 42 neuron pairs when standard whole cell recording methods were used[25], and therefore suspected that some critical cytoplasmic factor was being washed out of the cells. Accordingly, all our later experiments used the nystatin perforated patch technique to record from both pre- and postsynaptic neurons.

Figure 1 shows an experiment in which an action potential was repetitively fired (at 0.1 Hz) in one neuron, and the resulting synaptic current was recorded under voltage clamp in a neighbouring neuron (panel a). Panel b plots the amplitude of the current as a function of time during the experiment. For the first series of stimuli (before the arrow) the mean peak conductance was 1.62 nS, the standard deviation was 1.04 nS and on 8 out of 97 occasions the presynaptic action potential failed to evoke a postsynaptic response. At the arrow the bathing medium containing 10 mM Mg^{2+} (to prevent any possible potentiation during the test stimulation) was exchanged for one with no added Mg^{2+}, the postsynaptic neuron was switched from voltage clamp to current clamp, and the presynaptic neuron was tetanized three times for 2 s at 20 Hz. After this stimulation, the bathing solution with 10 mM Mg^{2+} was reintroduced, postsynaptic voltage clamp was re-established, and the 0.1 Hz stimulation of the presynaptic cell resumed. Following the tetanic stimulation, the mean synaptic current amplitude was 3.43 nS, the standard deviation 0.97 nS, and no failures in transmission occurred in 128 trials. The potentiation factor f in this case was $(3.43/1.62) = 2.1$. The amplitude histograms for pre- and post-tetanus synaptic current are shown in Fig. 2.

The following results argue in favour of the phenomenon in Fig. 1 being LTP. (1) The potentiation was long-lasting. For example, the e.p.s.c. in Fig. 1 was still at the same, elevated level 60 min after the tetanus. In two other cells the roughly threefold potentiation lasted for 80 and 90 min, respectively (at which point the cells were lost). (2) Potentiation could only be induced in a low Mg^{2+} concentration, as expected from the triggering characteristics of LTP[7,8]. (3) Potentiation was not an

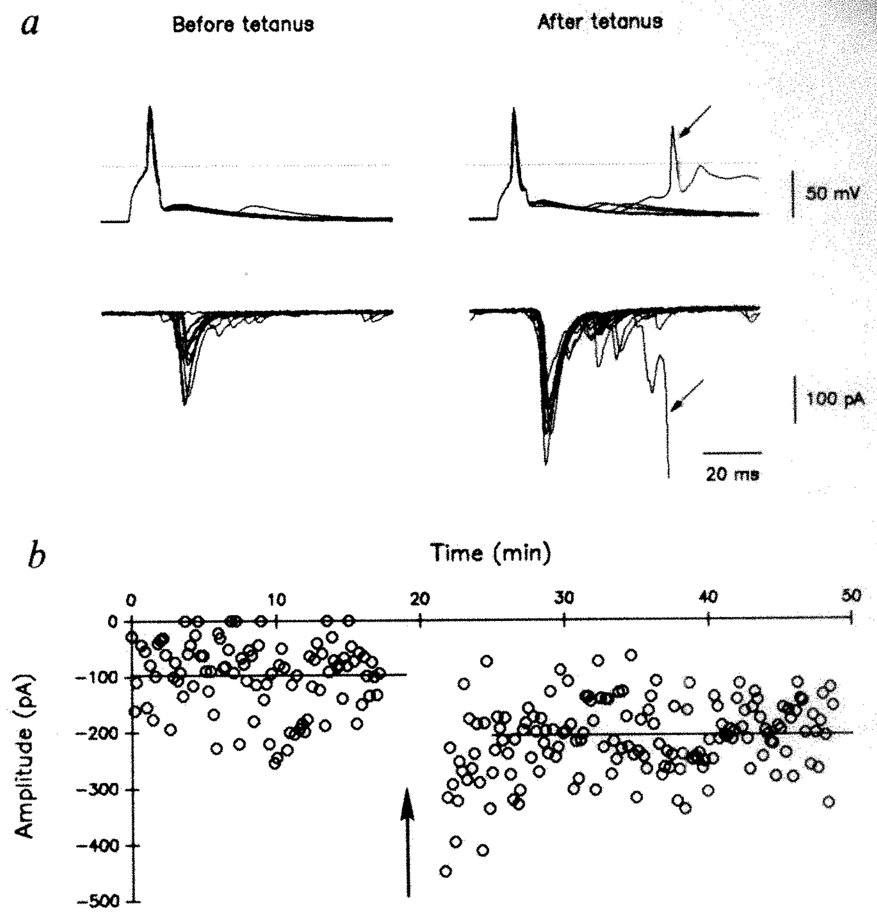

FIG. 1 Long-term potentiation of excitatory synaptic transmission between a pair of rat hippocampal neurons in dissociated cell culture. *a*, Superimposed action potentials recorded in the presynaptic cell (upper traces) and corresponding e.p.s.cs recorded simultaneously in the postsynaptic cell (lower traces). Left-hand panels were recorded shortly before, right-hand panels about 10 min after, a series of three 2-s tetani at 20 Hz separated by 30 s, performed with the postsynaptic cell in current clamp. All pre- and post-tetanus recordings were made with 10 mM Mg^{2+} solution (see below) in the bath; the tetani were delivered after briefly changing to a bath solution lacking Mg^{2+}. Action potentials were fired by injecting into the presynaptic cell a 10-ms 1 nA current step at 0.1 Hz. The presynaptic cell's resting membrane potential was near −60 mV; the postsynaptic cell was voltage clamped at −60 mV. Arrows in the right-hand panels indicate spontaneous polysynaptic activity that was recorded simultaneously in both cells (the current trace, lower panel, goes off-scale). Such activity was much more frequent after tetanus. Calibration bars refer to both right- and left-hand panels. The dotted lines in the upper panels indicate 0 mV. *b*, Plot of peak e.p.s.c. amplitude as a function of time for the same experiment as in *a*. The arrow indicates the time at which the sequence of tetani was applied. Immediately before this the bath was changed to a 0 mM Mg^{2+} solution, and then immediately afterward back to the control 10 mM Mg^{2+} solution. The straight lines through the data points represent the mean amplitudes over the time windows indicated by the horizontal positions of the lines.

METHODS. Pyramidal neurons from fields CA1 and CA3 of the hippocampi of newborn Long Evans rat pups were maintained in dissociated cell culture as previously described[21], except that 50 μM D-APV (D-2-amino-5-phosphonovaleric acid) was added to the culture medium after about a week to block possible potentiation due to spontaneous firing. For electrophysiology, the bath solution comprised (in mM) NaCl (137), KCl (5), $CaCl_2$ (3), $MgCl_2$ (10 or 0), D-glucose (10), HEPES-NaOH buffer (5), pH 7.3, osmolarity adjusted to 310 mOsm with sorbitol, to which was added 1 μM glycine, 1 μM strychnine and 100 μM picrotoxin. The tips of the patch electrodes were filled with a solution comprising (in mM) $KMeSO_4$ (150), KCl (5), EGTA (10), HEPES-KOH buffer (10), pH 7.2, and electrodes were back-filled with the same solution plus 100 μg ml^{-1} nystatin, diluted from a stock of 20 mg nystatin per ml of DMSO. Patch electrodes were Sylgarded and polished and had resistances of 1–3 MΩ with these solutions. After obtaining GΩ seals on a pair of cells, test pulses were applied until the access resistances stabilized (typically 30–50 min after forming the seals); access was checked for constancy throughout the experiment. Recordings were made with an Axopatch 1A, filter setting 1 kHz. The bath was perfused at about 1 ml min^{-1} (bath volume 1 ml). Electrode offset potentials were within 2 mV at the end of the experiment. All measurements were made at room temperature (23–25 °C).

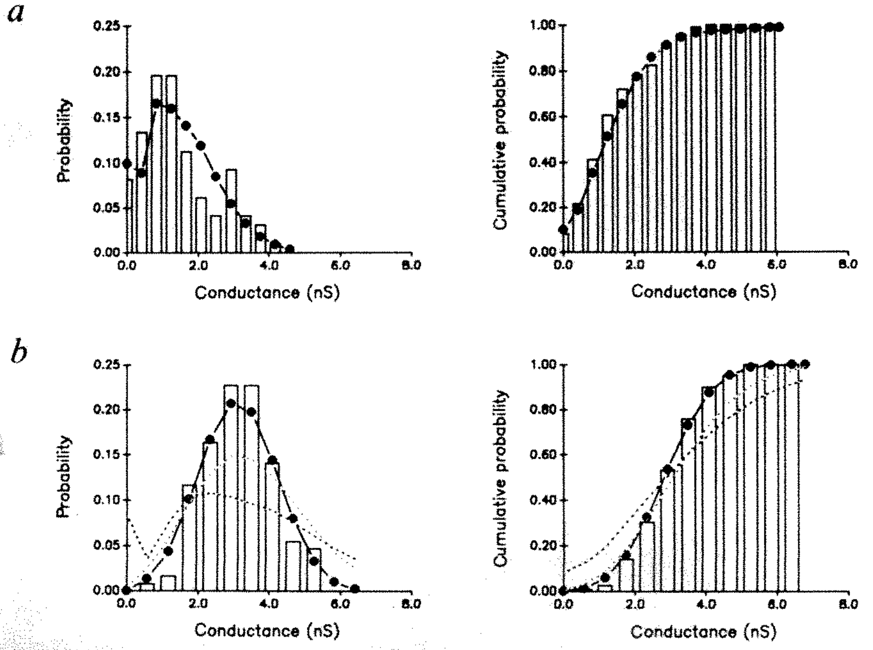

FIG. 2 Frequency histograms of e.p.s.c. conductance before and after inducing LTP are best fitted by assuming that LTP expression originates in an increase in release probability, *p*. *a*, Control (pre-tetanus) histograms. *b*, Post-tetanus histograms. (Same experiment as in Fig. 1.) In both *a* and *b* the right panel is the normalized cumulative histogram of the histogram in the left panel. In *a* the filled circles represent a binomial distribution fitted as described in the text. In *b* the filled circles were calculated from the distribution in *a* assuming that only *p* is scaled by the potentiation factor *f*, with no free parameters. The dotted line and the broken line were calculated assuming that the parameters *N* and *a*, respectively, are scaled by *f*.

artefact due to changing recording conditions. For example, the presynaptic action potential remained constant (Fig. 1*a*), and the bath was perfused at a continuous rate, preventing changes in the concentrations of possible modulatory substances[26].

Long-lasting potentiation was seen in 4 cell pairs out of 13 stable, connected pairs studied with the perforated patch technique. However, even in experiments in which the evoked e.p.s.c. was not potentiated, the above protocol usually caused a long-lasting increase in the general polysynaptic activity of cells in the culture. An example of this is visible in Fig. 1*a*, after tetanus (arrows). This is probably due to the potentiation of synapses other than those between the cell pair under study, and may arise in heterogeneity in the ability of synapses to support LTP[21].

Our strategy was to carry out a quantal analysis of the pre-tetanus responses then to test, without estimating additional parameters, three alternative mechanisms for the potentiation by predicting the amplitude histogram obtained after potentiation had developed. The potentiated response in Fig. 1 could be due to $f = 2.1$-fold scaling of N (the number of release sites), p (the quantal release probability) or a (the mean quantal size); as each of these factors enters the equation for the synaptic current size histograms in a different way, scaling each makes different predictions about the shape of the histograms. Thus we may determine which of the alternative theories best accounts for the data.

The probability of observing a synaptic current of a particular amplitude describing the amplitude histogram is given by the binomial distribution[14] suitably modified to take account of the observed variability in the m.e.p.s.c. size[20]. If a is taken to be the average value for the quantal size in culture (0.8 nS), the pre-tetanus binomial parameters N and p can be estimated from the standard equations for the mean $m = 1.62$ nS and variance $v = 1.073$ nS^2 of the amplitude histogram (Fig. 2*a*):

$$m = aNp$$

$$v = a^2Np(1-p) + a^2Npc_m^2.$$

The second term gives the contribution to the overall variance of fluctuations in the m.e.p.s.c. size; the coefficient of variation of m.e.p.s.cs is c_m and has an average value of 0.5 in culture and 0.42 in slices[20]. Solving these equations yields $N = 4.8$, $p = 0.42$. As N should be an integer, N was constrained to be 5 (rather than the calculated 4.8) and p was set to 0.37 (instead of 0.42).

The predicted and observed pre-tetanus histograms are shown superimposed in Fig. 2*a*; they are not significantly different at the 0.2 level (χ^2 goodness-of-fit test); that is, worse fits would occur by chance more than one time in five even if the histograms were generated by the same underlying mechanism. Although we could not in these experiments collect sufficient quantities of pre-tetanus data to carry out a very accurate quantal analysis, the fit exhibited in Fig. 2*a* confirms our previous conclusion[21] that the Del Castillo and Katz formalism, with the m.e.p.s.c. variability included, accounts for the observed amplitude histogram.

Figure 2*b* shows the result of assuming that the mechanism of LTP is an f-fold increase in the release probability p, with N and a unchanged; the predicted histogram that results is superimposed on the observed post-tetanus amplitude histogram. The predicted and observed histograms are not significantly different at $P < 0.2$. The two other alternative mechanisms, an f-fold increase in N or in a make predictions that differ from the observed histograms at $P < 0.05$ and 0.01 levels of significance, respectively (dotted and broken lines in Fig. 2*b*).

A convenient way to compare predicted and observed histograms uses the coefficient of variation (and its squared value h)

$$c = \sqrt{h} = \sqrt{v}/m$$

introduced in this context by Del Castillo and Katz[14], and modified here to take account of m.e.p.s.c. variability:

$$h(N, p) = (1 + c_m^2 - p)/(Np). \quad (1)$$

Note that a cancels in the right-hand side of this equation, so

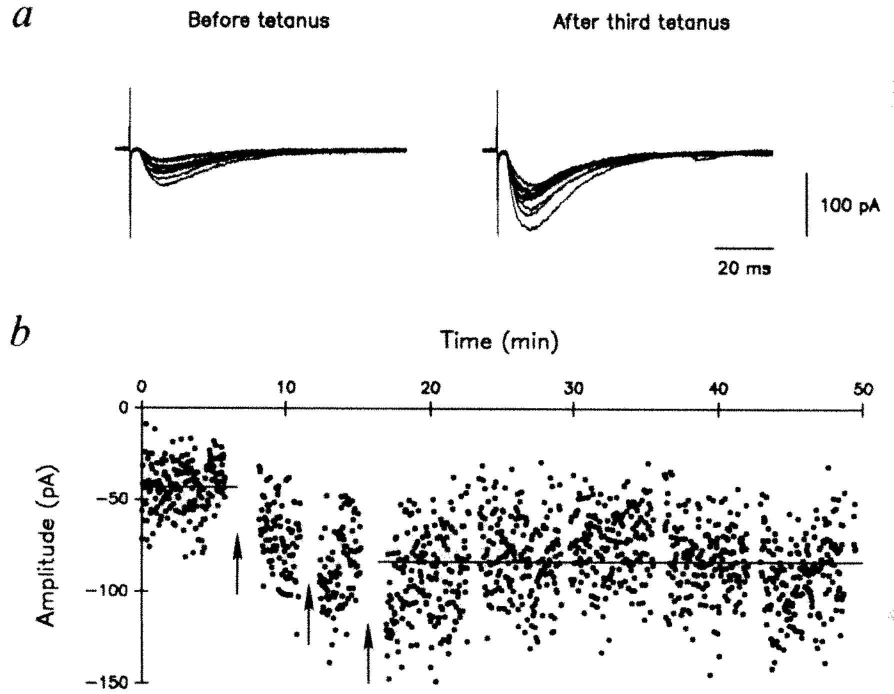

FIG. 3 Long-term potentiation of excitatory synaptic transmission in a patch-clamped hippocampal slice. *a*, Superimposed e.p.s.cs, elicited by stimulation of the Schaffer collateral pathway at 0.5 Hz, recorded from a cell in area CA1 before (left) and after (right) tetanus-induced LTP. The neuron was whole-cell voltage-clamped at the soma at −70 mV. Calibration bars refer to both panels. *b*, Plot of the amplitudes of evoked e.p.s.cs as a function of time during the experiment. At each arrow the patch amplifier was switched to current clamp mode and two 200-ms 100 Hz tetani were delivered 5 s apart. The amplifier was then returned to voltage clamp mode and testing resumed. The straight lines through the data points represent the mean amplitudes over the time windows indicated by the horizontal positions of the lines.

METHODS. Transverse slices (300–400 μm) were cut from the hippocampi of 14–21-day-old Long Evans rats using a vibratome, submerged and continuously perfused at 1–2 ml min^{-1} with oxygenated Krebs solution comprising (in mM) NaCl (120), KCl (3), MgCl$_2$ (1.2), CaCl$_2$ (2.5), NaHCO$_3$ (23), NaH$_2$PO$_4$ (1.2), D-glucose (11), to which 100 μM picrotoxin was added. Patch electrodes contained (in mM) Cs-methylsulphate (120), CsCl (5), EGTA (0.5), MgCl$_2$ (2), Mg-ATP (2), sorbitol (10), HEPES-CsOH buffer (10), pH 7.3. Gigaohm seals were obtained with unpolished electrodes (resistances 3–5 MΩ) on the somas of cells in region CA1 and recordings made in the whole-cell with an Axopatch 1A, filter setting 2 kHz. A monopolar stimulating electrode (35 μm diameter teflon-coated Pt-Ir wire) was positioned 200–300 μm away in *stratum radiatum* and the stimulus intensity set just above the level that gave mostly failures of synaptic transmission; this setting was subsequently left unchanged throughout the experiment. The stimulus duration for low frequency stimulation was 100 μs; for tetani it was 200 μs. Electrode offset potentials were within 2 mV at the end of the experiment. Experiments were performed at room temperature (23–25 °C).

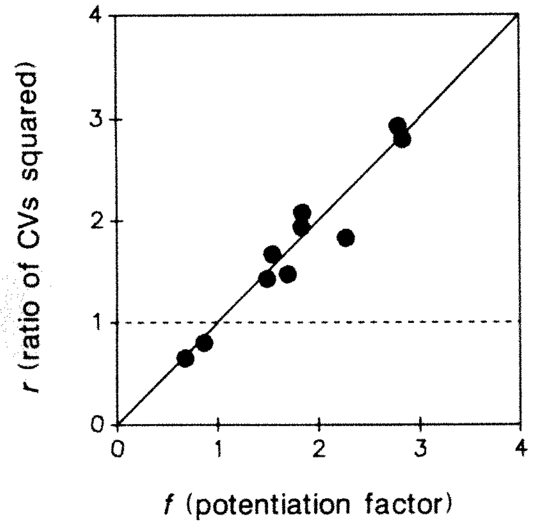

FIG. 4 A plot of r against f (defined in the text) for 10 different slice LTP experiments is best fitted by a model that locates the expression of LTP in the presynaptic terminal. The diagonal line is the prediction of a Poisson scheme in which LTP is expressed presynaptically, the horizontal line the prediction for postsynaptic expression. The two points for which f is less than unity were obtained from experiments in which 100 μM D-APV was present during the tetanus, and a long-lasting depression resulted.

that the value of h is not altered by a potentiation mechanism that operates through increases in a. According to this equation, if a increased f-fold, $h(N,p)=0.48$ (the pre-tetanus value), whereas $h(fN,p)=0.23$, and $h(N,fp)=0.12$. The observed value for h in this experiment was 0.08. This supports the above conclusion that LTP in culture involves an increase in p rather than in N or a. The same conclusion was reached in full quantal analyses of three other cell pairs in culture that displayed long-lasting potentiation.

Because the potentiation just described is enduring, and requires activation of synapses under conditions that permit calcium influx through the NMDA (N-methyl-D-aspartate) receptor channels, it is like LTP[7,8]. LTP is, however, normally studied in relatively intact hippocampal circuits, not dissociated cultures, so one cannot be sure that the mechanism discussed above operates in the same way as actual LTP. For this reason, we experimented with whole-cell clamped neurons in hippocampal slices, where the properties of LTP are well known.

Experiments like those just described, in which one records with the perforated patch technique from defined cell pairs, are difficult in the slice as one can only rarely find monosynaptic connections[16]. Accordingly, we used the conventional whole cell ruptured patch technique to record from single postsynaptic pyramidal neurons while repetitively exciting axons of the Schaffer collateral pathway with a small extracellular stimulating electrode[27,28]. An example is shown in Fig. 3. Panel *a* shows superimposed e.p.s.cs recorded while voltage clamping the neuron at −70 mV, measured before and after tetanus. Panel *b* plots the e.p.s.c. amplitude as a function of time during the experiment. Tetani were delivered at each of the arrows in Fig. 3*b* while maintaining the cell in current clamp. This protocol induced a long-lasting potentiation of the e.p.s.c.; the mean control amplitude was 43.3 pA, and the mean following three series of tetani was 83.8 pA.

In the above experiment the stimulus level was set just above the threshold for synaptic transmission. We knew, however, that multiple axonal inputs onto the postsynaptic cell were being stimulated. This situation complicates the quantal analysis[15].

Analysis in hippocampal slices

We have taken two approaches to extending our analysis to the hippocampal slice. First, we have used a procedure, described below, that takes account of the possible nonuniformity of the stimulated inputs to a neuron. Second, we have simply applied the standard equations of quantal analysis, and have relied upon goodness-of-fit tests to determine if the application of these equations is valid. These approaches are described in turn.

The first approach is best described by presenting a graph that plots a quantity r as a function of the factor f by which the mean e.p.s.c. size is potentiated. The quantity r, like f, is determined directly from the experimental data and is defined as the ratio

$$r = h/h_p$$

where h (defined earlier in equation (1)) is the squared value of the coefficient of variation before a tetanus and h_p is the corresponding quantity in the potentiated slice. In the limiting case where p is small (the Poisson limit), r is, according to equation (1), equal to f if the mechanism of LTP is an f-fold change in either N or p (presynaptic mechanism) and equal to 1 if LTP results from a scaling of a (postsynaptic mechanism). Figure 4 shows such an r/f plot for 10 slice experiments and unambiguously accords with the predictions of the presynaptic mechanism. Note that in two cases f was less than unity; in these experiments the tetanus was applied with 100 μM D-APV in the bath, which resulted in a long-lasting depression.

The r/f plot can be generalized to many situations with multiple inputs to a neuron in which a standard quantal analysis is not applicable. It can be shown in these cases that points will fall on or to the left of the diagonal ($r=f$) if the LTP mechanism is presynaptic (an increase in either N or p), and will fall on a horizontal line ($r=1$), or between that line and the diagonal, if the mechanism is postsynaptic (an increase in a). However, analysis reveals a special case for which the r/f plot does not correctly distinguish between a pre- and postsynaptic mechanism for LTP. Suppose that individual postsynaptic membranes could exist in only two states, 'on' and 'off'; for an 'off' synapse release of a quantum of neurotransmitter would result in essentially no response, and for an 'on' synapse, each quantum would give a standard-sized response. The mechanism of LTP in this model would be the postsynaptic switching (for example, by increasing the sensitivity of receptors in the postsynaptic membrane) from 'off' to 'on'. Such a switch would appear in our analysis to be an increase in N, and thus to be presynaptic, whereas in fact the site of LTP expression would be postsynaptic. From our slice experiments alone we would not be able to distinguish between the presynaptic mechanism and this particular postsynaptic mechanism. The observations in culture, however, are not consistent with the postsynaptic mechanism because LTP there resulted from an increase in the release probability p, not N.

We conclude that our observations in slices, like those in culture, are consistent with the expression of LTP being primarily presynaptic. Because points fall along the diagonal, indicating that release is described by the Poisson limit, we cannot here, as we could in some of the experiments on neuron pairs in culture, distinguish between effects on N and p.

An alternative way to test our conclusion is to carry out a full quantal analysis of transmission in these slice experiments: if the mechanism is indeed presynaptic and the quantal parameters sufficiently constant to give data points that fall along the diagonal in the r/f plots, then the distribution of synaptic current sizes should be well described by the standard equations of quantal analysis, and one should be able to predict the distribution of potentiated synaptic current sizes with the quantal parameters obtained from the unpotentiated distribution. If the conclusion is incorrect, then the observed distribution of synaptic current sizes should be a sum of Poisson distributions, and may not, in general, be adequately described by a single Poisson distribution.

Figure 5*a* shows a slice experiment in which the postsynaptic response for a small stimulus is plotted as a function of time

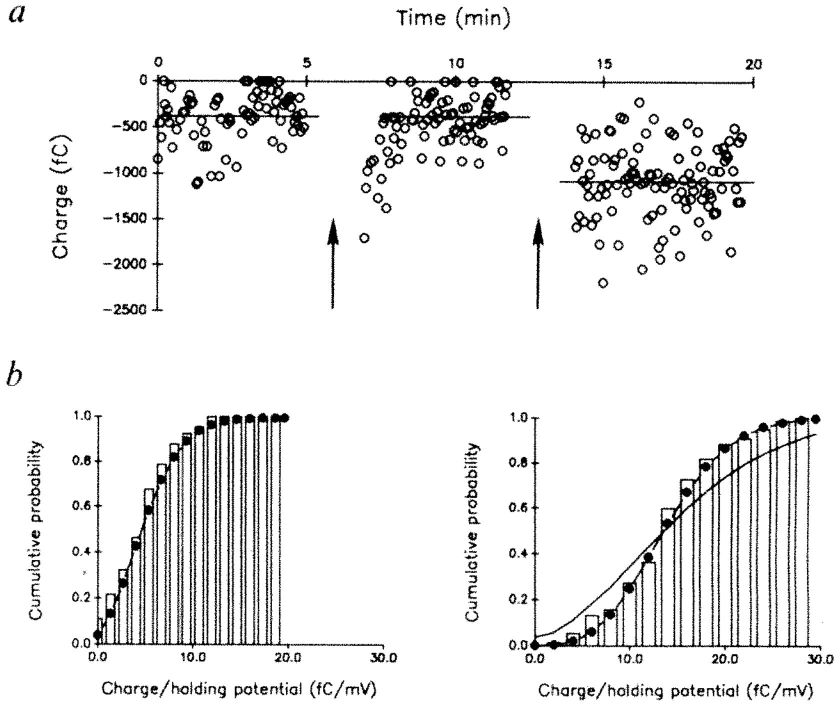

FIG. 5 Histograms of e.p.s.c. size in the slice are best fitted by a Poisson model in which LTP expression originates in a (presynaptic) increase in the mean quantal content, *Np*. *a*, Plot of the charge carried by evoked e.p.s.cs as a function of time during the experiment. Charge was calculated by integrating individual e.p.s.cs over an 80–90-ms time window starting at the foot of the e.p.s.c. Test stimuli were delivered at 0.5 Hz while voltage clamping the soma at −70 mV. At the first arrow, three trains of 200-ms 100 Hz tetani were delivered at 5-s intervals while still voltage clamping the soma at −70 mV. At the second arrow the same tetanus protocol was applied while the voltage clamping at −20 mV. The straight lines through the data points represent the mean amplitudes over the time windows indicated by the horizontal positions of the lines. *b*, Cumulative probability histograms derived from the data in *a* before (left) and after (right) inducing LTP. Note that the charge carried by the e.p.s.cs has been normalized by dividing by the holding potential (−70 mV). The filled circles on the left are a fit of the Poisson distribution to the experimental histogram. The filled circles on the right were calculated from the Poisson distribution assuming that LTP scales only *Np* by *f*, with no free parameters. The solid curve on the right is the prediction of a Poisson distribution assuming that only parameter *a* is scaled by *f*.

during the experiment. Note that charge, rather than amplitude, is measured because the former is a quantity less affected by cable attenuation in the dendrites[17,29]. At the point indicated by the first arrow, the inputs to the cell were stimulated repetitively but the postsynaptic neuron was maintained by the voltage clamp at −70 mV; the response did not potentiate after this procedure, as expected for LTP[3–6]. At the second arrow, the cell's inputs were again stimulated while the cell was held at −20 mV, a value that should maximize calcium influx through the NMDA receptor channels and produce LTP[7,8]. The mean response after this tetanus was potentiated by a factor $f = 2.84$. This particular experiment was chosen for illustration of the full quantal analysis because failures were apparent in the control records, which provides an additional check on the validity of the fitted parameters.

Figure 5*b* shows the histogram of observed synaptic responses for this experiment with superimposed predictions of the Poisson distribution, using appropriate values for *a* and *Np* estimated from the mean and variance of the raw data, and again taking into account the measured variance in m.e.p.s.c. size[20]. A χ^2 goodness-of-fit test detects no difference between these distributions at the 0.2 significance level. The quantal size estimated by the ratio variance/mean for this experiment is 2.1 fC mV^{-1} before (and after) the tetanus. For six experiments, the average quantal size was 1.35 ± 0.37 fC mV^{-1} before the tetanus and 1.41 ± 0.28 fC mV^{-1} in the potentiated slice (mean ± s.d.). These estimates should be compared with the mean value for *a* directly determined in slices of 1.5 fC mV^{-1} with a standard deviation of 0.5 fC mV^{-1} (ref. 20). The value for *a* estimated from the histogram is thus within the range of quantal sizes determined independently.

If the pre-tetanus value for *Np* is multiplied by *f*, the predicted histogram, superimposed on the experimental one after potentiation, is shown in Fig. 5*c*. Again the χ^2 goodness-of-fit test detects no difference between the observed and predicted histograms at the 0.2 significance level. If *a*, rather than *Np*, is multiplied by *f*, the predicted and observed histograms differ at the 0.01 significance level, so the differences are statistically different (Fig. 5*c*, smooth curve). Similar results were found in four other slice experiments in which we carried out full quantal analyses. We conclude that the potentiation mechanism is presynaptic, and that either *N* or *p* increases following the tetanus.

The longest LTP observed in our slice experiments is 98 min, and our conclusions hold up to that time. We cannot, however, exclude the possibility that slower developing phases of LTP use some different mechanism. Because our experiments were done at room temperature (23–25 °C), and we do not know the temperature coefficients of the various possible phases of LTP, we cannot even place limits of the validity of our conclusions for the hippocampus at body temperature. From just after the tetanus up to 1 h after, only a single phase of LTP was apparent in our experiments, and the statistical properties of the postsynaptic response were essentially invariant.

Discussion

Our conclusion is that the mechanism of LTP is presynaptic rather than postsynaptic, and probably involves *p* instead of *N*. We have not, however, attempted to place specific limits on the extent to which intermediate cases, in which two or three of the quantal parameters are affected by LTP, might hold. Specifically, we cannot exclude the possibility that some small fraction of LTP is postsynaptic, or that both *N* and *p* increase with the *p* effect predominating. Because the potentiation factor *f* enters squared if the LTP mechanism is postsynaptic and linearly if it is presynaptic, a large contribution from the postsynaptic site can be excluded. *N* as well as *p* could be affected significantly, and we would not be able to detect this.

Our conclusion that the expression of LTP is, under the conditions of our experiment, mainly presynaptic, directs attention to mechanisms whereby retrograde signals from the postsynaptic cell cause enhanced presynaptic release of neurotransmitter[13,30,31].

Note added in proof: Our observations and conclusions are in accord with those reported by R. Malinow and R. W. Tsien[32]. ☐

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LETTERS TO NATURE

Detection of diffuse interstellar bands in the infrared

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THE diffuse interstellar bands (DIBs) are spectral absorption features caused by interstellar matter but not identified with any known atomic or molecular lines. Since 1975, when 39 such bands were known[1], many more have been detected, and in 1988, about 80 were listed[2]. They range in frequency from visible (4,430 Å) to very near infrared wavelengths (8,650 Å) (ref. 3), but their carriers—the atoms or molecules that cause them—remain unknown. We have found two new DIBS in the near infrared, near 11,800 Å and 13,200 Å, in the spectra of reddened O, B and A stars. Their full widths at half maximum are 2.7 ± 0.3 Å and 4.0 ± 0.5 Å respectively, and their equivalent widths are 60 and 135 mÅ per magnitude of extinction. The wavelength of the second DIB is a factor of 1.5 longer than that of the longest wavelength band previously identified, which may be an important constraint on the nature of the carriers of these two bands.

Among the candidates for the carriers of the DIBs, carbonaceous molecules are particularly attractive because carbon is abundant and its bonds can survive the ultraviolet radiation in the diffuse interstellar medium. Several candidates have been proposed: carbon chains[4], polycyclic aromatic hydrocarbons (PAHs)[5,6], which are thought to be abundant in the interstellar medium, and fullerene ions[7,8]. Kroto[9] has shown theoretically that only a few such ions can form stable clusters, mainly C_{50}^+, C_{70}^+ and especially the most symmetric one C_{60}^+, a result in agreement with laboratory experiments[7]. Such species could be abundant in the interstellar medium and may be responsible for some of the DIBs. No spectroscopic data on C_{60}^+ are available but according to calculations using the Hückel model[8], C_{60}^+ has a ground-state transition at 4,500 Å (±20%) that is weakly allowed and could give rise to the observed DIB at 4,430 Å. The same calculations imply that there should also be a very strong DIB in the infrared[8] owing to an allowed transition at 1.2 μm (±20%). This led us to look for interstellar features in the near infrared.

Our observations were carried out on the 3.6-m Canada–France–Hawaii telescope at Mauna Kea using a Fourier-transform infrared spectrometer with a resolution of $\sim 10^4$ (resolution element 0.9 cm^{-1}). From Herbig[1], we selected reddened stars exhibiting DIBs in the visible. The stars studied are listed in Table 1—they have different spectral types as well as various values of colour excess, E(B–V). During the first run (September 1988), we studied two objects using the J, H and K filters and extracted two possible interstellar features in the J filter but none in the others. In the second run (November 1989), we observed four objects using only the J filter.

To remove most of the telluric absorptions (mainly H_2O absorption bands), we divided the spectrum of an object by the spectrum of a reference star. A good reference should be located at the same air mass as the object and also be of the same spectral type. Moreover, to allow for correct removal of stellar features without cancelling truly interstellar ones, the standard star should show no reddening. We tried to fulfil these criteria as much as possible. The remaining features in the ratio spectra are of three kinds: (1) incompletely removed telluric lines; (2) intrinsic stellar features that are not strictly compensated (for example, Fig. 1*A* shows He I features visible in the ratio of the spectra of object 6 and a reference of spectral type B1 III). Both these kinds of features can be identified because they are prominent in the raw spectrum of objects and references; (3) interstellar features. We consider a feature to be interstellar if it is present in the most reddened stars (applying a 3σ detection criterion) and absent in references, if its strength varies with E(B–V), and if its position from one line of sight to another can be explained by a reasonable Doppler shift. Typical interstellar clouds have a velocity dispersion of <50 km s^{-1}, which gives a maximum variation from one line of sight to another of 2 Å for a mean wavelength of 12,000 Å.

We found two such features. The stronger band lies between 13,174 Å (7,588.5 cm^{-1}) and 13,176 Å (7,587.6 cm^{-1}) (wavelengths λ are measured in air at standard temperature and pressure and wavenumbers σ are measured in vacuum: $\lambda(\text{Å}) = \sigma(\text{cm}^{-1}) \times 10^8/n$ where n is the index of refraction of air, 1.000273) and has a full width at half maximum (FWHM) of 4.0 ± 0.5 Å. The wavelength range of the weaker band is 11,797 Å

TABLE 1 Stars observed

Object	Name	Spectral type	Visual magnitude	J-filter magnitude	E(B–V)
1	BD + 40°4220	O7f	9.20	4.67	2.00
2	HD183143	B7Ia	6.87	3.56	1.28
3	HD194279	B1.5a	7.02	4.23	1.22
4	HD20041	A0Ia	5.92	4.06	0.73
5	HD223385	A3Ia	5.42	3.50	0.59
6	HD24398	B1Ib	2.85	2.65	0.34

(8,474.3 cm^{-1})-11,798 Å (8,473.9 cm^{-1}) and the FWHM is 2.7 ± 0.3 Å. We have not tried to correct these wavelengths for the velocities of the interstellar clouds in front of the stars.

Curves *b* and *c* in Fig. 1*B*, *C* show the two features in the ratio spectra of objects 1 and 2, respectively. Figure 1*Ba*, *Ca* show the ratios of references with other references giving an estimate of the remaining spurious features and Fig. 1*Bd*, *Cd* are the raw spectra of the references showing no clear structure at these wavelengths in unreddened stars. The shape of the two features seems slightly asymmetric owing to possible contributions from several clouds along the line of sight or to overlapping telluric bands that are not completely removed. The latter also introduces an error in the evaluation of the equivalent widths. To estimate this error, we have compared the ratios obtained with different references and the calculated uncertainties take this into account, as well as the uncertainty owing to noise.

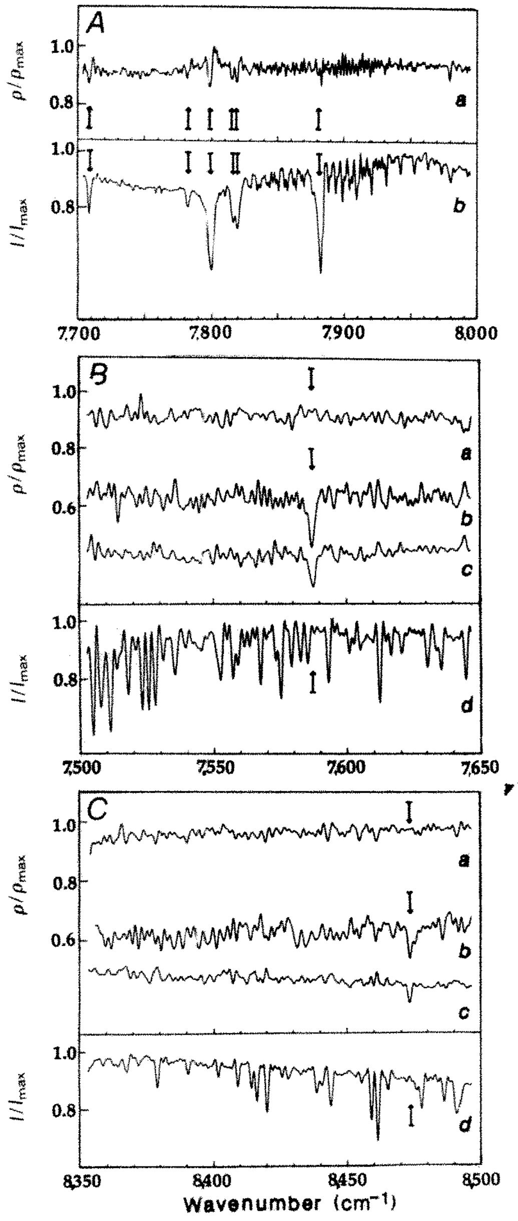

FIG. 1 Spectra with *A*, stellar features and *B*, diffuse interstellar features. *Aa* shows the incomplete removal of the He I lines when the spectrum from object HD24398 is divided by the spectrum of the reference star HD23180 (spectral type B1III), shown in *Ab*. *Ba* and *Ca* show the ratios of the spectra from some reference stars (HD33111 (spectral type A3III)/HD216627 (spectral type A3V) and HR1203 (B1Ib)/HR1122 (B5III)). *Bb* and *Bc* show the interstellar band at 1.32 μm for BD +40°4220 and HD183143. *Cb* and *Cc* show the interstellar band at 1.18 μm for BD +40°4220 and HD183143. *Bd* and *Cd* show that the DIBs at 1.32 μm and 1.18 μm are absent in the spectrum of reference star HD23180 and of the coadded references HR7924 (spectral type A1ae) and HR1122, respectively. Each spectrum is normalized at its maximum value. For clarity, the curves have been shifted vertically.

In Fig. 2, the two equivalent widths are plotted against E(B−V). The correlation coefficients are 0.91 and 0.71 and the equivalent widths are 135 and 60 mÅ per magnitude of visual extinction. The features for object 4 seem to be as strong as for objects towards which the extinction is twice as large. Herbig also observed this for the DIB at 5,780 Å.

In addition to a correlation with reddening, we checked that these features are probably not of stellar origin using a simple model of stellar atmosphere. For any given equivalent width and wavenumber, we obtain a condition of minimum abundance on the absorbing element assuming an oscillator strength ≤1, where we consider an element to be an atom with a certain degree of ionization and in a certain energy level. We searched tabulations of atomic and ionic transitions[10] but did not find any suitable candidate in a range of 5 cm^{-1} around the observed positions.

The two observed features are most likely not atomic or ionic transitions from simple atoms in the interstellar medium because all tabulated transitions around these two frequencies (±5 cm^{-1}) involve high energy levels, which cannot be sufficiently populated by the interstellar radiation field or by collisions.

What about molecular carriers? The observed transitions are certainly electronic because allowed vibrational transitions have low oscillator strengths and the position of the bands requires that they would arise from combination modes or overtones, which have a much weaker intensity. In the interstellar medium, neutral molecules are mostly in their ground state. For molecules fulfilling Platt's criterion[11] (such as those having $\Pi \rightarrow \Pi^*$ transition), the wavelength of the first allowed electronic transition from the ground state is proportional to the molecular size; such a transition in the infrared implies quite large molecules. This model[11] gives a linear size of 28 Å (25 Å) for a band at 7,588 cm^{-1} (8,474 cm^{-1}). For pericondensed (the most compact arrangements of aromatic rings) PAHs, which are expected to be abundant interstellar carbonaceous molecules and candidates for the carriers of the DIBs[5,6], such a dimension implies nearly 350 carbons per molecule[12], compatible with the size distribution of these molecules derived from infrared data[13]. But this result does not apply to radicals, which can have transitions at lower energies. Given that interstellar species can be partially ionized and dehydrogenated, and therefore can be radicals, the two transitions could arise from smaller species.

As mentioned above, calculations using the Hückel model imply that C_{60}^{+}, if responsible for the DIB at 4,430 Å, could provide a very strong absorption feature at 1.2 μm (±20%). The two detected bands are much weaker than expected (and the observed ratio of the oscillator strengths at 1.32 μm and 0.4430 μm is 2×10^{-2} whereas the expected ratio between an allowed and a forbidden transition should be much greater than unity). Although it is tempting to conclude that C_{60}^{+} cannot

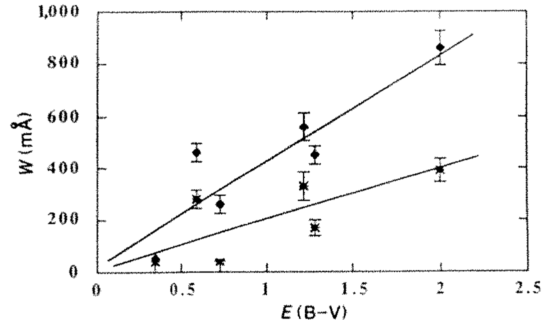

FIG. 2 Equivalent width of the 1.32-μm feature (diamonds) and the 1.18-μm feature (asterisks) plotted against selective extinction E(B−V).

account for the 4,430-Å band, the spectral range observed (the J band: 1.2 μm (±12%)) is not wide enough to cover fully the region in which the C_{60}^+ band might be expected owing to uncertainty on the position from calculations using the Hückel model. Experimental data are needed to try and constrain the abundance of C_{60}^+ in the interstellar medium.

Further studies are necessary to address, for example, whether or not there is a relationship between these two bands and the families of DIBs recently determined in the visible[14]. It is also important to determine whether these bands are present in molecular or diffuse clouds and if they pertain to neutral or ionized species. □

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Extending the power of powder diffraction for structure determination

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IN the modern techniques of high-resolution neutron and X-ray powder diffraction, an impressive amount of structural information may be obtained, allowing the refinement, using the Rietveld technique[1], of structures containing more than 100 positional parameters. Despite these technical advances and the renewed popularity of the technique, powder diffraction suffers from the inevitable loss of information imposed by the collapse of three dimensions of diffraction data onto the one dimension of a powder pattern. This can seriously frustrate the determination of unknown crystal structures. As a consequence of accidental or exact reflection overlap, only relatively few reflections may have uniquely determined intensities. (For example, the cubic reflections 550, 710 and 543 exactly overlap.) Here I present a maximum-entropy algorithm (previously discussed theoretically[2]) that evaluates, from first principles, the intensities of overlapping reflections in powder diffraction profiles. Indeed, a full three-dimensional reconstruction of the image may be retrieved when only ~20% of the reflections are uniquely determined. This precludes systems where the Patterson group is not the holosymmetric group (for example, the *4/m* symmetry where *hkl* and *khl* reflections are overlapping but not equivalent.) Nevertheless, for most crystal structures with unit cell volumes of less than 1,000 Å³, this method should be useful.

Crystallography is rooted in the mathematics of the Fourier transform. The structure factor $F(\mathbf{h})$ (in general a complex quantity) that is associated with reflection $\mathbf{h}$ is the Fourier coefficient of the atomic structure $\rho(\mathbf{x})$

$$F(\mathbf{h}) = \int_V \rho(\mathbf{x}) \exp(2\pi i \mathbf{h} \cdot \mathbf{x})\, d^3x$$

In a crystallography experiment the measured quantity is $|F(\mathbf{h})|^2 = F(\mathbf{h})F^*(\mathbf{h})$ and the phase information is lost. $|F(\mathbf{h})|^2$ is the Fourier component of the Patterson function $P(\mathbf{u})$, the autocorrelation function of the scattering density (the interatomic vector map of the crystal structure)

$$|F(\mathbf{h})|^2 = \frac{1}{2} \int_V P(\mathbf{u}) \cos(2\pi \mathbf{h} \cdot \mathbf{u})\, d^3\mathbf{u}$$

In a powder diffraction experiment many reflections may overlap and, in general, measured observations are of the form

$$\begin{aligned} G_i &= \sum_{n=1}^{N_i} J(\mathbf{h}_n) |F(\mathbf{h}_n)|^2 \\ &= \int_V P(\mathbf{u}) \left[\sum_{n=1}^{N} \left\{ \sum_{j=1}^{J(\mathbf{h}_n)} \cos(2\pi \mathbf{h}_{n_j} \cdot \mathbf{u}) \right\} \right] d^3u \qquad (1) \end{aligned}$$

where J is the number of symmetry equivalent reflections. Although the observations are no longer Fourier coefficients of any meaningful function, equation (1) is nevertheless linear and, if the Patterson map is divided into N_{pix} pixels may be expressed in the form

$$G_i = \sum_{k=1}^{N_{\text{pix}}} R_{ik} P_k \quad (i = 1, \ldots, N_{\text{obs}}) \qquad (2)$$

The coefficients R_{ik} correspond to the quantity inside the square brackets of equation (1). The method proposed here is to maximize the 'Patterson entropy'

$$S = -\sum_{k=1}^{N_{\text{pix}}} P_k \ln(P_k / Q_k) \qquad (3)$$

where Q_k is the *a priori* estimate (assumed flat) of the Patterson map, while maintaining agreement with the observed data D_i (standard deviation σ_i) through the χ^2 constraint

$$\sum_{i=1}^{N_{\text{obs}}} \frac{1}{\sigma_i^2} (D_i - G_i)^2 = N_{\text{obs}}$$

The maximum-entropy technique is a powerful method of image reconstruction that has been successfully applied to a number of different areas of research ranging from radio astronomy[3] to crystallography. The application of maximum-entropy to crystallography has been discussed by several authors (see, for example, refs 4 and 5). The above formulation of the maximum-entropy algorithm implicitly assumes that the scattering density is everywhere positive. Although this is true for X-ray diffraction analysis, negative Patterson densities may result from the analysis of neutron diffraction data. In such cases the Patterson map is divided into two arrays with intrinsically positive and negative contributions. Equations (2) and (3) are then recast as

$$G_i = \sum_{k=1}^{N_{\text{pix}}} R_{ik} (P_k^+ - P_k^-) \quad (i = 1, \ldots, N_{\text{obs}}) \qquad (2a)$$

$$S = -\sum_{k=1}^{N_{\text{pix}}} [P_k^+ \ln(P_k^+ / Q_k^+) + P_k^- \ln(P_k^- / Q_k^-)] \qquad (3a)$$

The final step in the full three-dimensional reconstruction of the diffraction data is achieved by calculating individual Fourier components of the maximum-entropy Patterson function and thus separating the intensities of overlapping reflections

$$|F(\mathbf{h}_n)|^2 = \frac{1}{2} \int_V P_{\text{ME}}(\mathbf{u}) \cos(2\pi \mathbf{h}_n \cdot \mathbf{u})\, d^3u \qquad (4)$$

As an example of the method, I present an analysis of medium-resolution neutron powder diffraction data. Rutile (TiO_2) was selected because its relatively high tetragonal symmetry ($P4_2/mnm$: $a = 4.5929$ Å, $c = 2.9581$ Å) and the medium resolution of the data set collected ensures a reasonable degree of reflection overlap and because the presence of positive (O) and negative (Ti) scattering centres tests the generality of the method.

Diffraction data from a 5-cm³ sample of rutile were obtained

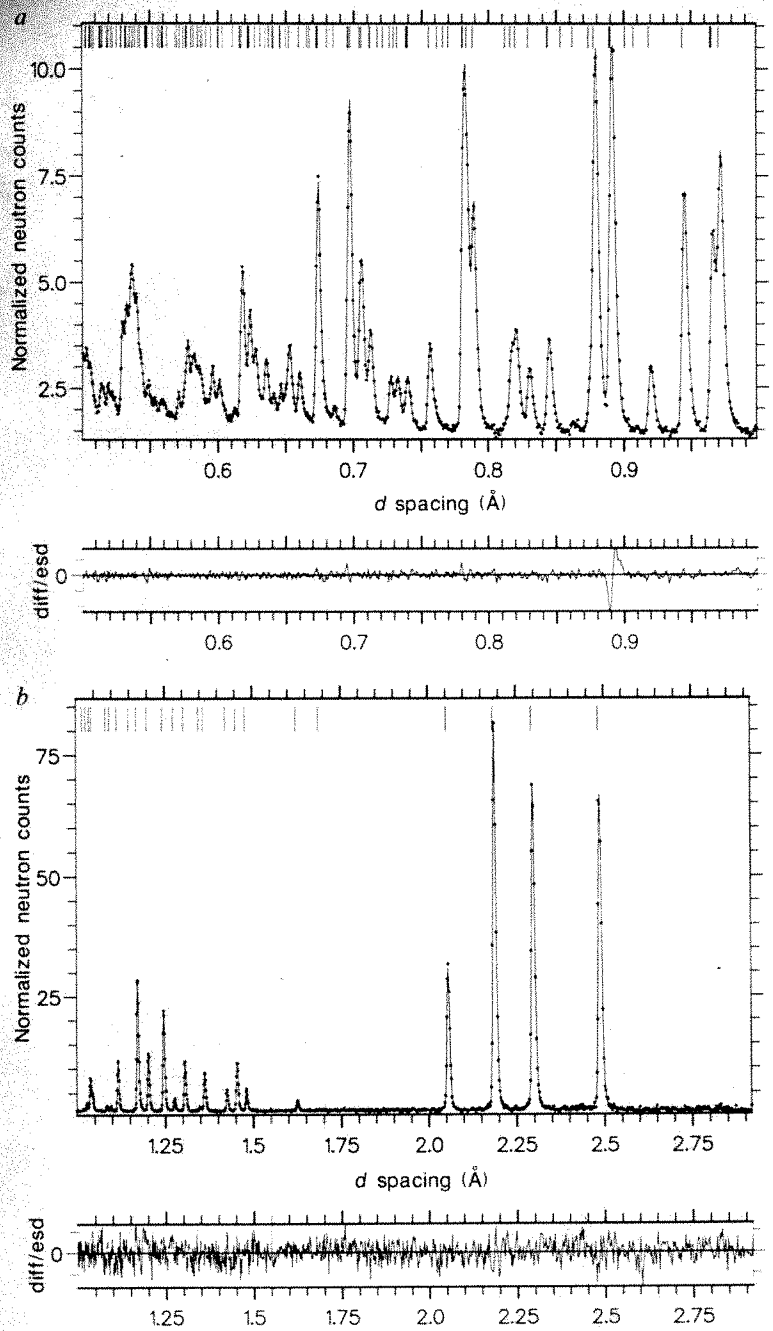

FIG. 1 The observed (dots), calculated (line) and difference profiles for TiO_2. The *d* spacings range from *a*, 0.5 to 1 Å and *b*, 1 to 2.9 Å. The positions of Bragg reflections are marked at the top and clearly indicate the severe peak overlap particularly at short *d* spacings; by eye, there are only 65 peaks in the full pattern. The difference profile is plotted as (observed − calculated profiles)/(standard deviation).

from the Polaris[6] diffractometer at ISIS in 2 h. After normalization, the profile was fitted by least squares refinement using the technique devised by Pawley[7]; in addition to the Bragg peak position (determined by the size of the unit cell) and the peak width variables, the intensities of individual Bragg reflections were refined as separate variables with the proviso that reflections that were close together (typically <10% of the full width at half maximum) were constrained to have equal structure-factor magnitudes. (This is the correct *a priori* Bayesian assumption.) 177 reflections were recorded in 130 clumps; 97 reflections were not overlapping. Comparison of the observed and calculated diffraction patterns (Fig. 1) indicates an excellent agreement ($\chi^2 = 1.371$). Figures 2*a–c* show sections of the Patterson map obtained by direct Fourier transformation of the equipartioned squares of the structure-factor magnitudes obtained from the Pawley refinement. Broad features dominate the maps with clear evidence of oscillations resulting from Fourier truncation errors and splitting of interatomic vector peaks caused by misapportioning of Bragg intensities. It is not possible to locate the interatomic vectors (listed in Table 1) with any degree of confidence. Using a uniform distribution, a maximum-entropy Patterson map consistent with the observed data was obtained in 64 iterations. The evolution of the entropy and χ^2 is presented in Fig. 3. The Patterson maps (Fig. 2*d–g*) obtained using the maximum-entropy algorithm contrast markedly with the Patterson maps derived by direct Fourier transformation. Sharp positive and negative features are located close to the correct

TABLE 1 Data from maximum-entropy Patterson map

	x (Å)	*y* (Å)	*z* (Å)	Δ (Å)	ME intensity	True intensity
Peak	0.0000	0.0000	0.0000	0.0000	1.671	1.147
Ti–O	0.3041	0.3041	0.0000	0.0010	−0.387	−0.399
O–O	0.3949	0.3949	0.0000	0.0064	0.325	0.337
Ti–Ti	0.5000	0.5000	0.5000	0.0000	0.166	0.118
Ti–O	0.1942	0.1942	0.5000	0.0014	−0.358	−0.399
O–O	0.5000	0.1110	0.5000	0.0014	0.667	0.673

Comparison of correct and observed (maximum-entropy Patterson map) interatomic vector positions and intensities for TiO_2.

Δ, fractional difference between ME and true interatomic vectors

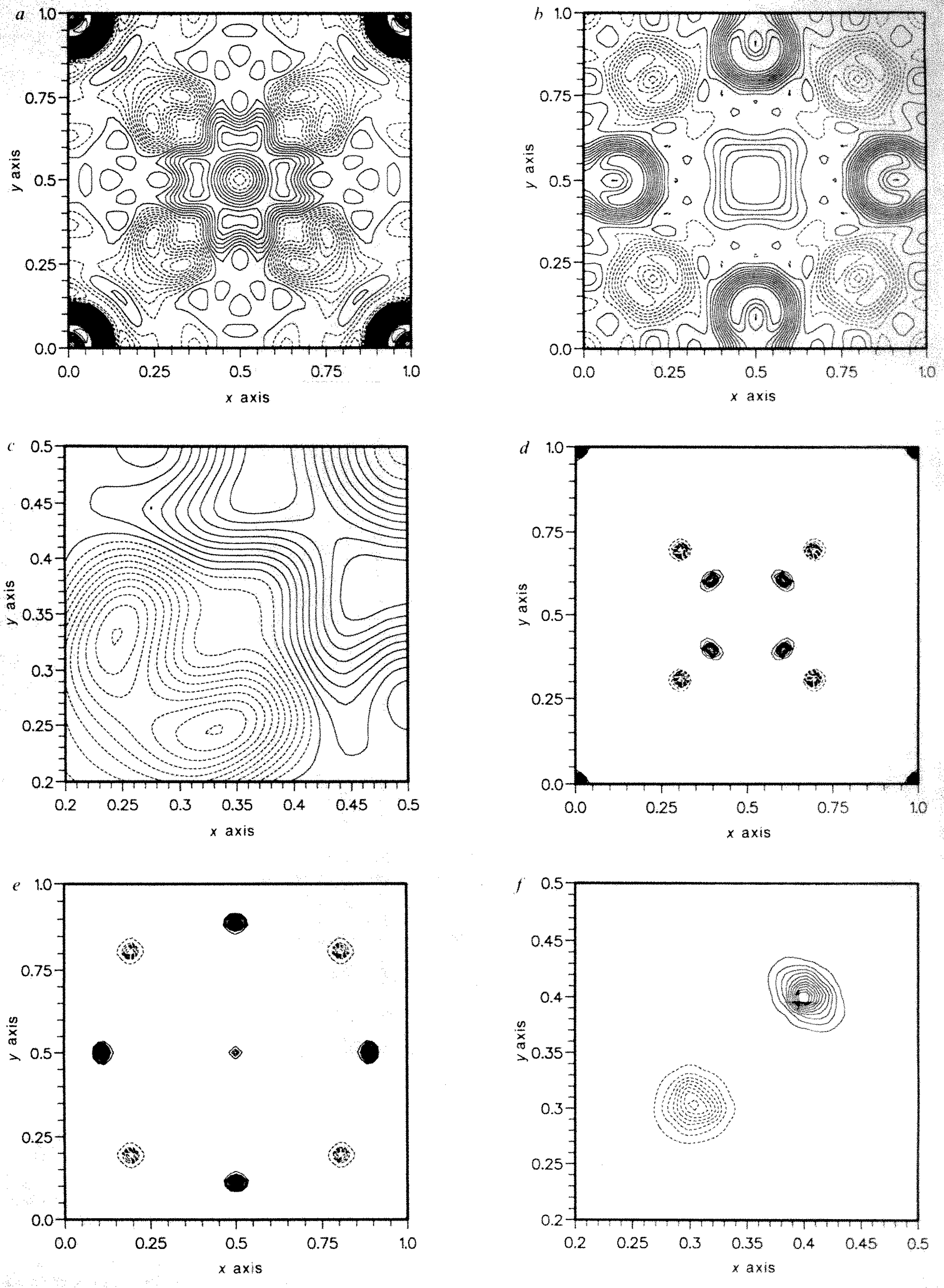

FIG. 2 *a–c*, Sections of the Patterson map synthesized by Fourier transformation of the squares of the structure factor magnitudes obtained from the Pawley-type profile refinement of TiO_2 and subsequent averaging over a sphere of diameter 0.5 Å. The first two maps range over the full *xy* plane at *a*, $z=0$ and *b*, $z=\frac{1}{2}$. *c* is a magnification of *a* for $0.2<x<0.5$ and $0.2<y<0.5$. *d–f*, Sections of the Patterson map obtained by maximum entropy reconstruction from the observed clumps of Bragg intensities. The ranges in *d–f* correspond to those of *a–c*.

interatomic vector positions. Table 1 shows that the typical deviation between observed and correct vector positions is $\sim 0.0015a = 0.007$ Å, a full order of magnitude less than the pixel resolution ($a/60 \approx 0.08$ Å) and almost two orders of magnitude less than the minimum d spacing measured (0.487 Å). The oxygen fractional coordinate calculated from the maximum-entropy Patterson map is 0.3047, essentially identical to the correct value of 0.3048. Moreover, the intensities of these interatomic vectors are in good agreement with expected values (Table 1) and there is even some evidence of the anisotropy of the thermal motion of the oxygen atoms (Fig. 2f). The algorithm has produced an optimally sharpened Patterson map and thus opens up a productive route for the solution of crystal structures.

Evaluation of the individual squared magnitudes of the structure factor was performed by back-transformation of the maximum-entropy Patterson map (for a full list of structure factors see Supplementary Information). A comparison of the following R factors shows that there is a clear superiority in the $|F(\mathbf{h})|^2$ derived from the maximum-entropy algorithm over the *a priori* equipartioned values.

$$R_{\mathrm{EQ}} = \left[\sum^{N_F} w(\mathbf{h})(F(\mathbf{h})_{\mathrm{EQ}} - F(\mathbf{h}))^2 / \sum^{N_F} w(\mathbf{h})F(\mathbf{h})^2 \right]^{1/2} = 34.0\%$$

$$R_{\mathrm{ME}} = \left[\sum^{N_F} w(\mathbf{h})(F(\mathbf{h})_{\mathrm{ME}} - F(\mathbf{h}))^2 / \sum^{N_F} w(\mathbf{h})F(\mathbf{h})^2 \right]^{1/2} = 14.1\%$$

$$R_{\mathrm{EX}} = \left[N_F / \sum^{N_F} w(\mathbf{h})F(\mathbf{h})^2 \right]^{1/2} = 6.5\%$$

R_{EQ} and R_{ME} are the R factors for the $|F(\mathbf{h})|$ obtained from the refinement of the Pawley profile and from the maximum-entropy algorithm, respectively, and R_{EX} is the expected R factor based on statistical errors. N_F is the number of different $|F(\mathbf{h})|$ and $w(\mathbf{h})$ is the inverse square of the estimated standard deviation of $|F(\mathbf{h})|$. Moreover, for all clumps of reflections the method correctly discriminates between strong and weak intensities, albeit usually with a slight reduced contrast than for the true values. Indeed, the $|F(\mathbf{h})|^2$ derived from the maximum-entropy algorithm for intensity clumps containing up to five reflections agree well with true values even at short d spacings close to the limits of the diffraction data. A further indication of the success with which the maximum-entropy algorithm has optimally combined the intensity information is provided by consideration of the sign of $|F(\mathbf{h})|^2$. Although the least-squares profile refinement has yielded negative values for some $|F(\mathbf{h})|^2$, all 177 $|F(\mathbf{h})|^2$ obtained by back-transformation of the maximum-entropy Patterson map are positive quantities. In general, therefore, the $|F(\mathbf{h})|^2$ obtained by the maximum-entropy method can be used to increase the potential for success of a direct-methods approach to structure solution using powder data.

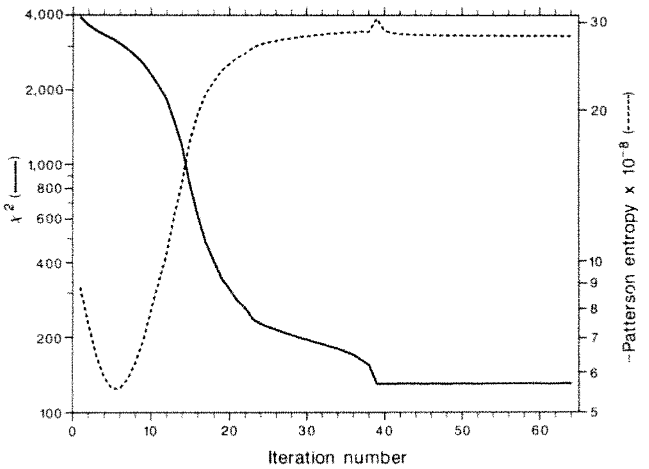

FIG. 3 Evolution of the entropy and χ^2 goodness of fit for the construction of the maximum-entropy Patterson map. (The negative value of the Patterson entropy is plotted as it is an intrinsically negative property.) The entropy decreases initially as the Patterson map changes from the flat uniform distribution to one consistent with the observed data ($\chi^2 = 130$) after 39 iterations. Once agreement with the observed diffraction intensities has been obtained the entropy increases to its maximum value consistent with the χ^2 constraint.

It is worth considering two broader implications of the maximum-entropy formalism that may have important ramifications for *ab initio* structure solution. The Patterson maps produced may be regarded as being optimally sharpened and thus be the closest approach to a true interatomic vector map. Although computationally expensive, this has broad implications for Patterson methods and will assist in the current revival[8] of the technique as an important alternative to direct methods of structure solution[9,10]. Furthermore, the formalism uses the information content contained in negative scattering factors. Many aspects of direct methods of structure solution from X-ray data rely implicitly on the inherent positivity of the electron scattering density. This poses concerns for neutron diffraction data where the scattering density may be both positive and negative and, indeed, weakens the power of direct methods in such situations. Here, the negative scattering density is a bonus. Consider, for example, a molecule in an asymmetric unit of a unit cell and containing a single atom with a negative scattering length. The negative peaks in the maximum-entropy Patterson map only correspond to vectors between the negative scatterer and all the other atoms. Such a situation bears a close resemblance to isomorphous replacement[11,12] and anomalous dispersion[12] methods. Examination of the negative Patterson features uniquely defines the location and orientation of the molecule with respect to the negatively scattering atom. ☐

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SUPPLEMENTARY INFORMATION. Requests should be addressed to the London editorial office of *Nature*.

Sensitivity of regional climates to localized precipitation in global models

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ATMOSPHERIC general circulation climate models are valuable tools for investigating the effects that large-scale perturbations (for example, increasing greenhouse gases[1], volcanic dust in the atmosphere[2], deforestation[3,4] and desertification[5]) might have on climate. Designed for global-scale climate simulations and having coarse spatial resolutions (from ~250 km (ref. 6) to 800 km (ref. 7)) such models are now being used to evaluate climatological and hydrological quantities at or near the land surface and at subcontinental scales[8–10]. They are also being used to provide input data for high-resolution simulations, predicting, for example, the

effects of increasing atmospheric CO_2 at regional scales[11]. Here we show that continental surface climatologies and climate change predictions for tropical forest ecotypes derived from general circulation models may be very sensitive to slight modifications in the land-surface/atmosphere coupling. Specifically our results indicate that improving the realism of the areal distribution of precipitation alters the balance between runoff and evaporation. In the case of a tropical forest, we find that by modifying the area over which rainfall is distributed, the surface climatology is changed from an evaporation-dominated regime to one dominated by runoff. Climate studies using near-surface output from such simulations may therefore be misleading.

The land surface is an essential component in atmospheric general circulation models (AGCMs). A series of sensitivity experiments[12] has shown that the simulation of the Earth's climate is affected by the land surface[3,8] and its representation[9,13]. In the past decade several models for parameterizing the land surface in AGCMs have been designed[4,14,15]. The latest include detailed soil and canopy models which, under the observed climatological forcing, realistically simulate surface-atmosphere energy fluxes, temperatures and the water balance for specific locations[3,9]. Although these land surface models are realistic, the atmospheric quantities output by the AGCM are not always compatible with those required by the land surface models. For instance, virtually all current AGCMs predict the precipitation rate as a grid-element average, that is, a single value for precipitation is calculated for each grid element at each timestep. Thus both large-scale (such as frontal) and small-scale (such as convective) precipitation is assumed to fall uniformly over the entire grid element. In the case of large-scale precipitation this may be satisfactory (particularly for models with spatial resolutions of ~300 × 300 km). In the case of small-scale precipitation, however, this grid-average assumption leads to a loss of physical realism. Specifically, current AGCMs predict drizzle across entire grid areas rather than intense convective precipitation concentrated in much smaller areas within grids. In the former situation a much greater proportion of the rainfall will be intercepted by the vegetation canopy and subsequently re-evaporated, whereas in the latter, more realistic situation, throughfall and hence runoff will be much greater. This modification in the partitioning of precipitation between runoff and evaporation has the potential to affect the energy and hydrological balance of the land surface. It may also cause systematic changes in the predictions by AGCMs once feedbacks are incorporated. These effects will be most pronounced in regions where dense vegetation exists and where convective precipitation is common, for example, in tropical forest areas.

Tropical convective precipitation can produce point-measured precipitation intensities of $10\ \mathrm{mm\,h^{-1}}$ but the spatial extent of such storms is only $\sim 100\ \mathrm{km^2}$. At this intensity, a canopy would saturate rapidly leading to leaf drip and therefore a large proportion of the precipitation would reach the soil surface where it would either infiltrate or run off. In an AGCM, convective precipitation is spread over the entire grid element. Assuming grid areas of $100{,}000\ \mathrm{km^2}$ ($\sim 3° \times 3°$) and a precipitation intensity of $10\ \mathrm{mm\,h^{-1}}$ leads to a precipitation intensity over the entire grid element of $\sim 10^{-2}\ \mathrm{mm\,h^{-1}}$. In this case the canopy would intercept and retain virtually all the precipitation, contrary to observation.

The evaporation of precipitation intercepted by a canopy occurs very rapidly in comparison to the evaporation of water that infiltrates the soil surface. The low precipitation intensities 'predicted' by AGCMs could therefore lead to fundamentally misleading hydrological simulations because precipitation recycling from the surface to the atmosphere, in any AGCM incorporating a vegetation canopy, will be overestimated at the expense of increasing soil moisture or runoff. To overcome this problem it is important to account for both the spatial extent and intensity of precipitation in AGCMs that incorporate the new generation of land surface schemes.

Here we investigate, using the Biosphere-Atmosphere Transfer Scheme (BATS)[15], a method of retaining the spatial extent and intensity of precipitation for AGCMs suggested elsewhere[16,17]. Throughout this discussion water fluxes are in units of $\mathrm{kg\,m^{-2}\,s^{-1}}$ ($1\ \mathrm{kg\,m^{-2}\,s^{-1}} = 1\ \mathrm{mm\,s^{-1}}$). It is possible to derive an expression for surface runoff[16] R_{surf} and canopy drip[17] R_{drip} by assuming that the local precipitation rate (over a fraction μ of the grid element) is described by a decaying exponential probability distribution. We note that although subgridscale variations exist in R_{surf} and R_{drip} within a timestep, knowledge of the distribution of local precipitation is not propagated—at the end of a timestep the soil moisture and intercepted water are assumed to be distributed uniformly within the grid element. This is clearly inconsistent with the definition of the net flux of water at the surface, P_s, but no method yet exists to account for subgridscale variations in intercepted water and soil moisture in AGCMs. Expressions for R_{surf} and R_{drip} are given by

$$R_{\mathrm{surf}} = P_s \exp\left(\frac{-\mu F_s}{P_s}\right) \tag{1}$$

where F_s is the maximum surface infiltration rate, assumed constant over the grid element. Similarly, the canopy drip rate is

$$R_{\mathrm{drip}} = P_c \exp\left(\frac{-\mu F_c}{P_c}\right) \tag{2}$$

where P_c is the precipitation intercepted by the canopy and F_c is the maximum canopy infiltration rate, assumed constant over the grid element. F_c is defined as the difference between the canopy storage capacity and the water stored on the canopy, divided by the model timestep.

In the standard version of BATS, R_{surf} is given by

$$R_{\mathrm{surf}} = \beta^4 P_s \tag{3}$$

where β is a depth-weighted ratio of the soil wetness. Equation (3) predicts $R_{\mathrm{surf}} > 0$ when any water falls to the surface. The canopy drip is usually determined by the storage capacity S ($\mathrm{kg\,m^{-2}}$). In a timestep Δt when the mass of intercepted water per unit leaf area C ($\mathrm{kg\,m^{-2}}$) on the canopy exceeds S, all water in excess of S falls to the surface

$$R_{\mathrm{drip}} = (C - S)/\Delta t \tag{4}$$

Here we replace these standard formulae by equations (1) and (2).

Using the modified version of BATS in a stand-alone mode[10] (that is, uncoupled from the GCM), and prescribing atmospheric forcing (including precipitation, air temperature, solar radiation, downward near-infrared radiation and wind speed) with seasonal and diurnal cycles from climatological estimates[18], the model was integrated for a two-year simulation to investigate the sensitivity of the land surface to changes in the definition of μ. Here we report results of the second year of integration (these results are independent of initial conditions) for a tropical forest, with μ set to 1.0 (rainfall over the whole grid element), 0.5 and 0.1. The results from the control simulation using the standard version of BATS are also included. We consider tropical forest and tropical rainfall because the dense canopy and the high leaf-area index typical of this ecotype leads to a high sensitivity to the type (and here the spatial extent) of precipitation simulated by AGCMs and because of the number of Amazonian deforestation experiments conducted using AGCMs[3,4,19]. These results are, however, likely to be appropriate to all regions where precipitation falls at spatial scales less than the resolution of the AGCM.

Figure 1 shows four sets of histograms of precipitation, evaporation and runoff for the four simulations. In each case the monthly total precipitation is identical, as is the atmospheric forcing but the fraction of the grid square over which the precipitation is prescribed to fall is variable.

The control simulation using the standard BATS model (Fig. 1*a*) shows that the seasonal variation in evaporation is limited,

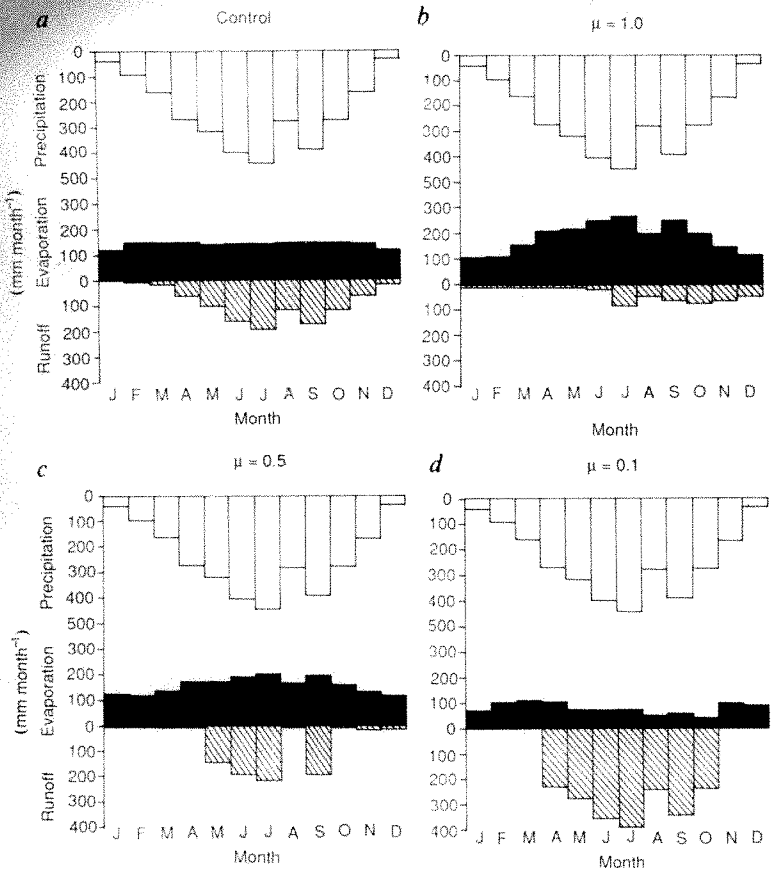

FIG. 1 Results from the second year of four 2-yr stand-alone simulations showing the seasonal variation in the precipitation (open bars), evaporation rates (solid bars) and runoff (hatched bars) for *a*, the control simulation (standard BATS without the μ-parameterization), *b*, BATS plus the μ-parameterization with $\mu = 1.0$; *c*, $\mu = 0.5$; *d*, $\mu = 0.1$. The precipitation forcing varies from 34 mm month^{-1} in December to 440 mm month^{-1} in July. The air temperature varies seasonally and diurnally, with the annual maximum in March–April (301 K) and the minimum in July–August (298 K) and a diurnal temperature range of 3 K. The wind velocity (at the height of the canopy) is constant at 3 m s^{-1} and the atmospheric pressure is constant at 1,000 hPa. Solar radiation varies seasonally and diurnally and is reduced after rainfall in an attempt to simulation the gross effects of cloud cover.

with the precipitation in excess of evaporation during the summer months (JJA) forming runoff. BATS incorporating the μ-parameterization (replacing equations (3) and (4) by equations (1) and (2)) produces very different simulations. Figure 1*b* ($\mu = 1.0$) shows much higher evaporation fluxes and very little runoff. For $\mu = 1.0$, the precipitation is distributed over the entire grid square leading to relatively low intensities. The dense canopy of the tropical forest intercepts much of the rainfall leading to higher monthly evaporation, but much lower runoff rates.

Although the spatial extent of precipitation in Fig. 1*a*, *b* is the same (distributed over the entire grid element, although non-uniform in Fig. 1*b*) it is clear that the resulting simulation of evaporation and runoff is quite different because the actual formulations of R_{surf} and R_{drip} are different. This leads to a change in the amount of precipitation that is intercepted and evaporated or that reaches the soil surface. The differences between Fig. 1*a*, *b* show how sensitive the simulation of the partitioning of precipitation between runoff and evaporation is to minor changes in the formulation of the land surface in AGCMs.

As μ is reduced from 1.0, the effective precipitation intensity is increased and interception rates are reduced, leading to lower evaporation and higher runoff. Figure 1*c* shows the case for $\mu = 0.5$. Evaporation shows some dependence on season (although less than in Fig. 1*b*) and high runoff during the summer months. Figure 1*c* shows negligible runoff in April, October and November, however, in contrast to Fig. 1*a*. Finally, Fig. 1*d* shows the simulation for $\mu = 0.1$. Here, precipitation is concentrated over 10% of the grid element, hence its effective intensity is relatively high. Figure 1*d* shows that there is relatively little evaporation except in February, March, April, November and December when evaporation rates are comparable with the minimum rates in Fig. 1*a*–*c*. The runoff simulated with $\mu = 0.1$ is dramatically higher than in the previous simulations. The high precipitation intensities lead to very low interception and high runoff rates for seven months of the year. In this case the canopy becomes saturated rapidly in a precipitation event, leading to high throughfall and subsequently large runoff rates.

The change from Fig. 1*a* or Fig. 1*b* to Fig. 1*d* is somewhat similar (decreased evaporation and increased runoff) to modelled results of tropical deforestation[3] although the only disturbance is in the spatial distribution of a constant precipitation amount falling on unchanged tropical forest. This implies that recent estimates of the impact on climate of tropical deforestation[3,4,19] are inconsistent because two[4,19] include some description of subgridscale precipitation and two[3,19] include a canopy parameterization. It is probably unrealistic simply to incorporate a μ-parameterization without considering very carefully the likely impact on the hydrology, which is a function of the canopy scheme. The relative and combined importance of subgridscale precipitation and vegetation must be more thoroughly investigated.

The quantitative values in the histograms of runoff and evaporation in Fig. 1 are of less importance (because minor changes in the land surface parameterization or in the prescribed atmospheric forcing would alter magnitudes) than the relative shapes of the seasonal distributions. The differences in the seasonal distributions shown in Fig. 1*a*–*d* are of considerable importance to potential users of AGCM simulations for quantities at or near the land surface. Figure 1 shows clearly that the surface hydrological climatology is highly sensitive to atmospheric 'forcing', in this case precipitation. Simply by changing the area over which precipitation is distributed, the surface climatology can be changed from an evaporation-dominated regime (Fig. 1*b*) to one dominated by runoff (Fig. 1*d*).

By prescribing atmospheric forcing, feedback effects between the surface and the atmosphere are prevented. As it is not obvious what direction feedbacks would take, it is unclear whether the simulations presented here are amplifications of the fully interactive result, or whether the results are damped. It is therefore necessary to analyse the μ-type parameterization in an AGCM before developing the methodology further.

These simulations suggest that it may not be possible to improve the simulation of climate at the land surface by improving only the land surface models. Advances in land surface modelling must be made in tandem with improvements in the coupling between the land surface and atmosphere. The addition of vegetation and soil processes to AGCMs improves physical realism, but it also has the potential to increase the sensitivity of the surface to the atmosphere. If incorporation of these more complete land surface submodels is considered desirable then the simulation of precipitation, including its subgridscale variability, must be improved. We do not necessarily claim improved accuracy for any of the regimes illustrated in Fig. 1, because observational evidence is not yet sufficiently accurate to validate any particular simulation. Rather we point out that including the effects of spatial heterogeneity in precipitation increases the physical realism of the model simulations and therefore its consequences must be fully investigated.

The distribution of precipitation within grid elements needs to be incorporated into the AGCMs that include vegetation. Its calculation is fairly straightforward[20] but even specifying precipitation intensities of large-scale and small-scale precipitation events as different but constant values[16,17] would improve the simulation of near-surface quantities. This approach is dangerous, however, because a constant value for μ could lead to a climatology dependent on the value chosen for μ. Figure 1*a*–*d* implies that using AGCM simulations of near-surface variables from models that incorporate a parameterization of vegetation, but do not consider subgridscale variability of precipitation, are misleading. Our results indicate that using results of AGCMs for regional-scale impact studies of, for example, greenhouse warming or deforestation might be very misleading unless the limitations of the model are clearly understood. □

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Partly pedogenic origin of magnetic variations in Chinese loess

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QUATERNARY sequences of interbedded aeolian silts (loess) and buried soils (palaeosols) potentially provide one of the best terrestrial records of past climates[1–5]. Magnetic susceptibility variations in loess and palaeosol sequences from China are strongly correlated with climate-induced fluctuations of oxygen isotope ratios in deep-sea sediments[2–4,6–8]. As an explanation of this correlation, it has been suggested that the recorded variations in magnetic susceptibility depend primarily on the degree of dilution by non-magnetic bulk loess constituents of a uniform magnetic assemblage derived from remote but unknown sources[6–8]. It has also been suggested that such a model, involving changes in the accumulation rate of loess superimposed on a constant flux of magnetic particles, provides a relative geological timescale through the control of loess deposition by astronomically modulated climate fluctuations[7]. Here we report a preliminary test of this model using rock magnetic properties especially sensitive to variations in magnetic grain size. Our results show that palaeosols are characterized by much finer magnetic grain size assemblages than are the intervening loess units. This suggests that a simple model based on constant magnetic influx and dilution by variable amounts of non-magnetic loess is inadequate. Our magnetic measurements establish the close comparability of the fine grained magnetic minerals in the palaeosol samples to those in contemporary soils and thus point to a partially pedogenic origin for the magnetic mineral assemblages in the palaeosols.

Here we present results obtained from two groups of samples. One (Fig. 1) consists of 17 samples of late Pleistocene loess and a palaeosol from Xifeng, Gansu (35.7° N, 107.6° E). The other (Fig. 2*a*, *b*) consists of samples taken from eight successive palaeosols and the loess units above and below them in the upper part of a Quaternary loess section near Lanzhou, Gansu (36.1° N, 103.8° E). Magnetic measurements were made on air-dried, disaggregated and weighed subsamples.

Several recent studies describe the instrumentation used for magnetic measurements and outline the way in which they may be interpreted[9–11]. These studies show how rock magnetic measurements, which reflect variations in magnetic mineralogy and grain size, can be used to characterize soils, dusts and sediments, to differentiate them and to establish their origins. Here the results from Xifeng (Fig. 1) are plotted in stratigraphic sequence, whereas those from the Lanzhou section (Fig. 2*a*, *b*) are presented as bivariate plots.

Figure 1 shows that field and laboratory measurements of the profile of the magnetic susceptibility are in excellent agreement, and that the latter are only slightly affected by expressing the results on a carbonate-free basis. The magnetic susceptibilities in palaeosol horizons are over three times higher and the χ_{fd} and χ_{ARM} values, which are strongly correlated, generally five to seven times higher than those in the loess. Moreover, variations in the normalized quotients $\chi_{\text{fd}}(\%)$ and $\chi_{\text{ARM}}/\text{SIRM}$ are positively correlated and in SIRM/χ negatively correlated with those in bulk magnetic susceptibility. These variations must therefore reflect differences between the samples in the relative proportions of different magnetic components, and cannot be caused simply by changes in the degree of dilution by non-magnetic constituents in the samples. More specifically, variations in $\chi_{\text{ARM}}/\text{SIRM}$ can be explained only in terms of the behaviour of the remanence-carrying minerals and cannot be affected by paramagnetic or diamagnetic components.

Measurements of IRM acquisition (not shown here) confirm that all samples from both sections attain 92–97% of the SIRM in a forward field of 300 mT. Therefore we conclude that the magnetic properties of these samples are dominated by ferrimagnetic (for example, magnetite-type) behaviour and that variations between the samples in the relative importance of imperfect anti-ferromagnets (such as haematite) will not alone account for the large differences in rock magnetic properties encountered.

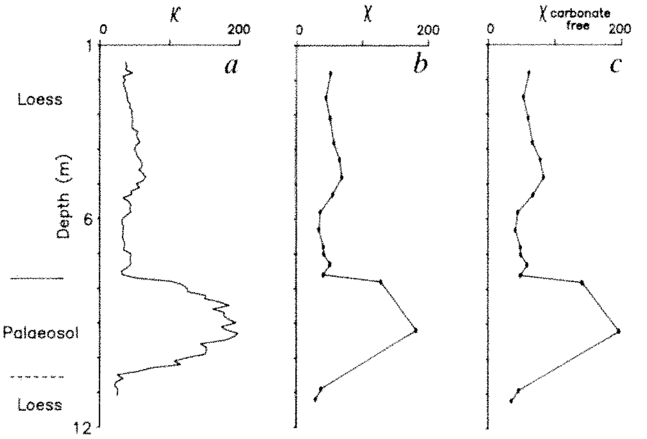
Many studies have shown that variations in ferrimagnetic grain size and thus domain structure have an important influence on the range of the magnetic parameters we have measured[12-16]. Maher[17] has demonstrated that in dispersed very-fine-grained synthetic magnetite samples with grain-interaction characteristics comparable to those in natural samples, two normalized properties, $\chi_{\rm fd}(\%)$ and $\chi_{\rm ARM}/\rm SIRM$, are especially responsive to variations in the magnetic grain size. Although based on simple assemblages of synthetic magnetite, her observations are consistent with a wide range of data from natural samples which include more complex mineral assemblages[9-11,18-21].

In our data, these two properties show a high degree of correlation. Palaeosol samples are characterized by $\chi_{\rm fd}(\%)$ values up to 8-10%, and $\chi_{\rm ARM}/\rm SIRM$ values up to $60\text{-}80\times 10^{-5}\ \rm A^{-1}\,m$. The intervening loess samples have lower values; $\chi_{\rm fd}(\%)$ is rarely over 5% and $\chi_{\rm ARM}/\rm SIRM$ is rarely over $35\times 10^{-5}\ \rm A^{-1}\,m$.

In Maher's New MT series of synthetic samples[17], maximum $\chi_{\rm fd}(\%)$ values of 7-11% occur in samples with grain sizes around the transition from stable single domain (SSD) to superparamagnetic (SP) behaviour, and only samples with mean grain diameters of 0.016-0.036 μm yield values over 2.5%. Peak values of $\chi_{\rm ARM}/\rm SIRM$ occur in the true SSD range, with values over $60\times 10^{-5}\ \rm A^{-1}\,m$ only in samples with mean grain diameters <0.07 μm. Magnetic grain assemblages that span the SP/SSD transition are the only ones with high values for both parameters.

From these observations we infer that the palaeosol horizons in both sections are relatively enriched in ferrimagnetic grains around the SP/SSD transition. These grains are finer than those responsible for the magnetic properties of the intervening loess. The recorded variations in $\rm SIRM/\chi$ reflect the same shifts in magnetic grain size. The reciprocal relationship between $\chi_{\rm ARM}/\rm SIRM$ and $\chi_{\rm fd}(\%)$ on the one hand (Fig. 2*a*), and $\rm SIRM/\chi$ on the other (Fig. 2*b*), arises because grains with sizes above the SP/SSD boundary have relatively low χ and high SIRM, whereas true SP grains exhibit high χ and zero SIRM. Lower $\rm SIRM/\chi$ quotients are thus indicative of the finer grains, and vice versa. The magnetic properties of the palaeosols compare well with those of many contemporary soils. For example, the majority of $\chi_{\rm fd}(\%)$ and $\chi_{\rm ARM}/\rm SIRM$ values for soils developed on contrasted lithologies in the Chesapeake Bay area[11] range from 7% to 13% and from 40 to $120\times 10^{-5}\ \rm A^{-1}\,m$ respectively. We conclude that significant proportions of the fine grained ferrimagnets characteristic of these palaeosols are pedogenic in origin and have been formed *in situ*.

Magnetic enhancement in topsoil has long been recognized[22-25]. The effects of fire and the *in situ* formation of maghemite and/or magnetite under soil-forming conditions are thought to be the dominant mechanisms involved[9,22-25]. Recent studies have demonstrated that in both natural and simulated soil environments magnetite can be formed through inorganic processes[26,27]. Meanwhile magnetotactic bacteria[28] have been found in modern soils[29]. The secondary magnetic phases, however formed in soils, have been shown to survive for long



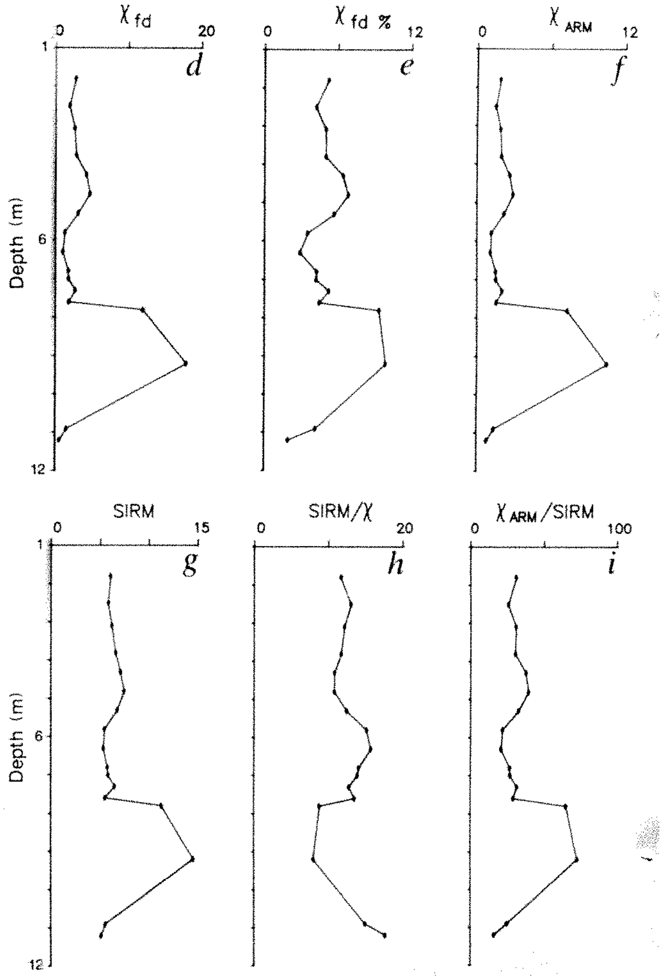


FIG. 1 Profiles of the magnetic properties of the Xifeng section. *a*, Volume magnetic susceptibility, κ (Bartington meter reading, in SI units), measured at 10-cm intervals in the field with the ferrite-probe attachment to the Bartington magnetic susceptibility meter M.S.2. This provides a simple and rapid way of detecting variations in magnetic concentrations. *b*, Mass-specific (dry-weight based) magnetic susceptibility, χ ($10^{-8}\ \rm m^3\,kg^{-1}$), measured in the laboratory. *c*, Similar to *b*, but expressed on a carbonate-free basis, $\chi_{\rm carbonate\,free}$ ($10^{-8}\ \rm m^3\,kg^{-1}$). *d*, Frequency-dependent magnetic susceptibility, $\chi_{\rm fd}$ ($10^{-8}\ \rm m^3\,kg^{-1}$), the difference in magnetic susceptibility measured at low and high frequencies (0.47 kHz and 4.7 kHz). Only magnetic grains close to the stable single domain (SSD)/superparamagnetic (SP) transition (~0.02 μm in the isodiameteric magnetite) contribute significantly to this quantity. *e*, Similar to *d* but expressed as a percentage of the low-frequency magnetic susceptibility, $\chi_{\rm fd}(\%)$, to provide an estimate of the relative proportion of total susceptibility contributed by transitional SSD/SP grains in each sample. *f*, Anhysteretic remanent magnetization (ARM), acquired in an a.c. field smoothly decreasing from 100 mT with a superimposed d.c. field of 0.04 mT, expressed as ARM per unit field, $\chi_{\rm ARM}$ ($10^{-6}\ \rm m^3\,kg^{-1}$). ARM values are highest in SSD grains with diameters between ~0.02 and 0.1 μm. *g*, Saturation isothermal remanent magnetization, SIRM ($10^{-3}\ \rm A\,m^2\,kg^{-1}$), acquired in a d.c. field of 1 T at room temperature, includes contributions from all the remanence-carrying magnetic minerals in the sample. *h*, $\rm SIRM/\chi$ ($\rm kA\,m^{-1}$). *i*, $\chi_{\rm ARM}/\rm SIRM$ ($10^{-5}\ \rm A^{-1}\,m$). The $\chi_{\rm ARM}$ and $\chi_{\rm fd}$ variations confirm the relatively high concentrations of fine SSD and SP grains in the palaeosol, the $\chi_{\rm fd}(\%)$ and $\chi_{\rm ARM}/\rm SIRM$ values establish their greater importance in the palaeosol as a proportion of the total magnetic mineral assemblage.

periods under favourable conditions[9,25,30,31]. The magnetic susceptibility of loess seems to be mainly, but probably not completely (see below), due to aeolian detrital magnetic minerals of larger mean grain size.

In addition to the contrast in magnetic properties between the palaeosol and loess, there are variations in both concentration-dependent and concentration-normalized properties within each. For example, the $\chi_{fd}(\%)$ for loess is ~2–5% (Fig. 1*e*) at Xifeng and 0.6–3% (Fig. 2*a*) at Lanzhou, whereas preliminary studies on samples from a site closer to desert areas to the north yield $\chi_{fd}(\%)$ values rarely above 1% (L.P.Z. unpublished data). This may reflect differences in provenance[5,19,32], varying distance from source areas[5,32] or some *in situ* pedogenic transformation during loess accumulation. Several studies[9,19] of contemporary dusts and aerosols suggest that variations in the magnetic properties of their primary detrital components are likely to reflect provenance-related differences rather than changes in volcanic or cosmic fluxes. Furthermore, in the Lanzhou samples, there is a wide range of variation in magnetic properties among the palaeosol samples although we observe no overall temporal trend (Fig. 2*a*, *b*). The variations may reflect differential magnetic enhancement resulting from differences in past pedoenvironments. At sites where parent materials, topography and climate were comparable[33], such a mechanism could give rise to the parallel variations of magnetic susceptibility as previously observed[6–8].

In so far as these and more detailed studies in China (B. A. Maher and R. Thompson, manuscript in preparation) and elsewhere[34,35] point to the role of pedogenesis and of possible source-related variations in controlling the magnetic record in loess sections, they replace the idealized constant magnetic influx-dilution model with a more complex one which could give more information about palaeoenvironment once fundamental problems of loess chronology are satisfactorily resolved. □

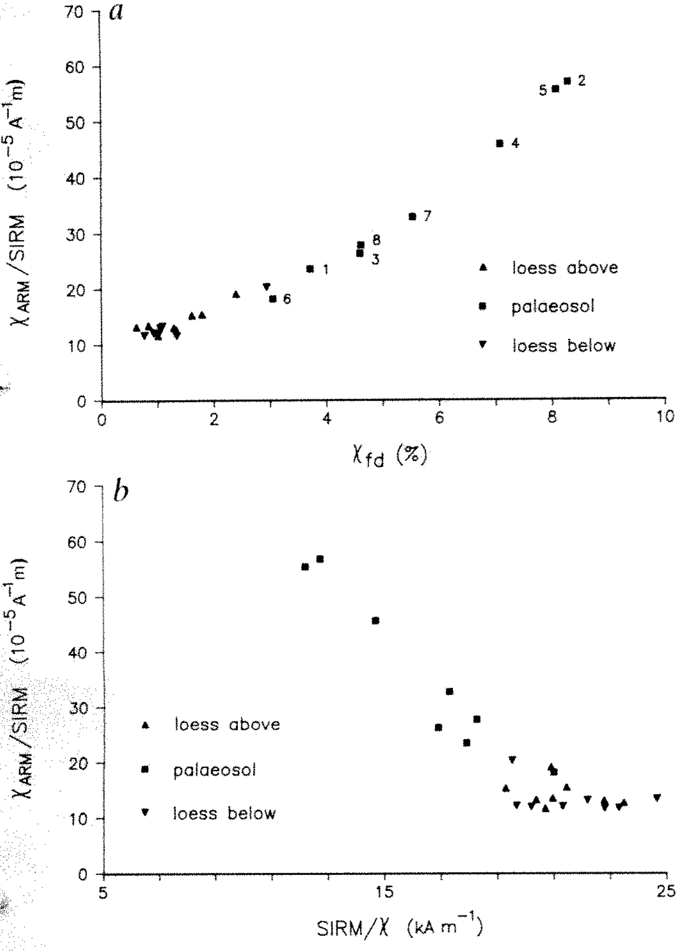


FIG. 2 Plots of *a*, χ_{ARM}/SIRM against $\chi_{fd}(\%)$ and *b*, χ_{ARM}/SIRM against SIRM/χ for the Lanzhou samples. A clear relationship between these two normalized properties that differentiate the loess and palaeosol samples in terms of magnetic grain-size proportions is shown in *a*. The two plots together support the view that SIRM/χ values in these samples, as in Xifeng section, are depressed in the palaeosols owing to their finer magnetic grain size.

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Differential denudation and flexural isostasy in formation of rifted-margin upwarps

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MARGINAL upwarps are common features of rifted continental margins[1,2], but tectonic models of the evolution of rifted margins have not adequately explained their form, or their persistence along some margins more than 100 Myr after continental rupture. Marginal upwarps not only significantly influence the geomorphological evolution of rifted margins and their adjacent continental interiors, but are also important in determining patterns of offshore sedimentation. Here we show that the contrast in denudation rates between the evolving coastal flanks of rifted margins and their interior hinterlands can promote significant marginal upwarps if the lithosphere responds flexurally to the resulting differential

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unloading[2,3]. Using data for the western margin of southern Africa, we demonstrate that upwarps of 600 m with respect to the adjacent continental interior can be generated by this process independently of the mechanics of rifting.

Although the morphology of rifted continental margins is highly variable, two main types of mature rifted margin (>60 Myr old) can be identified: (1) low-lying margins, such as southern Australia, which rise gradually from the coast to a low interior plain (low-elevation rifted margins); (2) steeply rising margins in which the coast is commonly separated from an often relatively high continental interior by a single major escarpment which has retreated inland[1] (high-elevation rifted margins). A typical feature of high-elevation rifted margins, such as those of southwestern Africa, eastern Brazil and eastern Australia, is the gradual decline in mean elevation inland of the escarpment, or series of escarpments (Fig. 1*a*). This gives rise to a rim of high terrain, or marginal upwarp, typically 300–900 m high and 50–300 km wide which separates the coast from the adjacent continental interior.

Marginal upwarps are also characteristic of young rifted margins, and in this setting they are usually referred to as rift-flank uplifts. Several models have been proposed to explain their development. Thermal effects[4,5] are time-dependent and decay with a time constant of ~60 Myr (ref. 6) and are therefore not applicable to upwarps associated with mature margins. Other models, including the regional compensation of lithospheric unloading[7,8] and depth-dependent extension[9,10] during rifting can generate some residual rift-flank surface uplift, but in each case its amplitude is reduced by sediment loading within the rift. More generally, marginal upwarps along mature rifted margins cannot be adequately explained by models based on dynamic models of rifting because the axis of maximum surface uplift along most mature rifted margins is now located 100 km or more from the hinge line marking the boundary between rifted and unrifted continental lithosphere. Underplating models[11] alone are also incapable of accounting for marginal upwarps because the domal uplifts generated by underplating create broad topographic swells of significantly greater extent than those characteristic of mature rifted margins[12]. Our proposed mechanism for the creation of marginal upwarps on mature rifted margins focuses on the isostatic response of the lithosphere to the contrasting rates of denudation that prevail on the coastal and inland flanks of the marked topographic discontinuity present along high-elevation rifted margins. The fact that such a discontinuity is found on both young rifted margins (such as those bordering the Red Sea[13]) and mature rifted margins indicates that it is a persistent feature of margin evolution.

Before rifting, the topography of supercontinents is probably one of predominantly low local relief owing to prolonged denudation, although regional elevation may be relatively high in areas remote from pre-rifting coastlines where local channel gradients, and hence rates of denudation, are low. In addition, underplating associated with rifting may generate a regionally extensive increase in elevation along some rifted margins soon after rifting begins[12]. Continental rupture will create significantly lower base levels along newly formed high-elevation rifted margins, and consequently two distinct denudational systems will be established, separated by a retreating major escarpment. This is illustrated by the western margin of southern Africa where

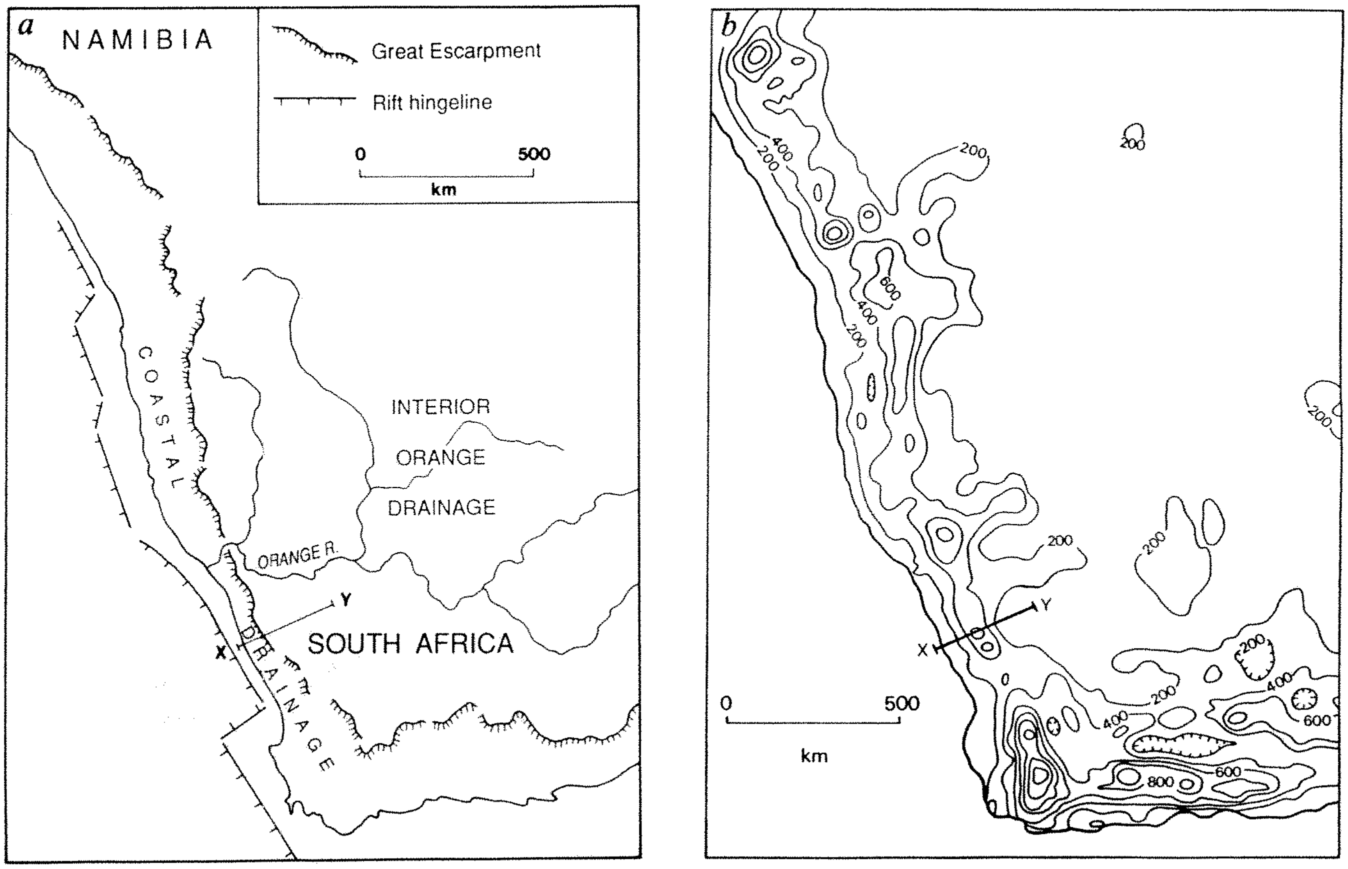

FIG. 1 Major morphological features (*a*) and variations in local relief (*b*) along the western margin of southern Africa. Local relief (maximum – minimum elevation) contours (m) have been generalized from local relief values derived from the 10-min digital topographic data set from the National Geophysical Data Center, Boulder, Colorado. The modelled topographic profile illustrated in Fig. 2 is shown by X–Y line on both maps.

the high local relief along the coast associated with high slope and channel gradients promotes relatively high denudation rates, whereas the very low local relief inland of the Great Escarpment gives rise to low denudation rates, although the regional elevation is relatively high (generally >1,000 m) (Fig. 1*b*). Along this margin the prominence of the escarpment is locally emphasized by the strong lithological control exerted by certain units in the Karoo sequence.

This denudational model is supported by several kinds of evidence. The overall depth of post-rifting denudation averaged over all Atlantic-draining catchments in southern Africa has been estimated to be ~1,800 m on the basis of offshore sediment volume data[14]. Apatite fission-track data indicate upwards of 2.5 km of post-rifting denudation along the margin[15] and therefore denudation rates in the interior must have been significantly lower. Fission-track analyses from other rifted margins also indicate significant post-rifting denudation oceanward of their escarpments[13,16]. Furthermore, given the former greater westward extent of the Karoo Basin[17], the exposure of older Karoo strata and basement rocks along the western margin of southern Africa also indicates significant depths of erosion, whereas the preservation of younger Karoo sediments and Cenozoic weathering profiles indicates generally low rates of post-rifting denudation in the interior.

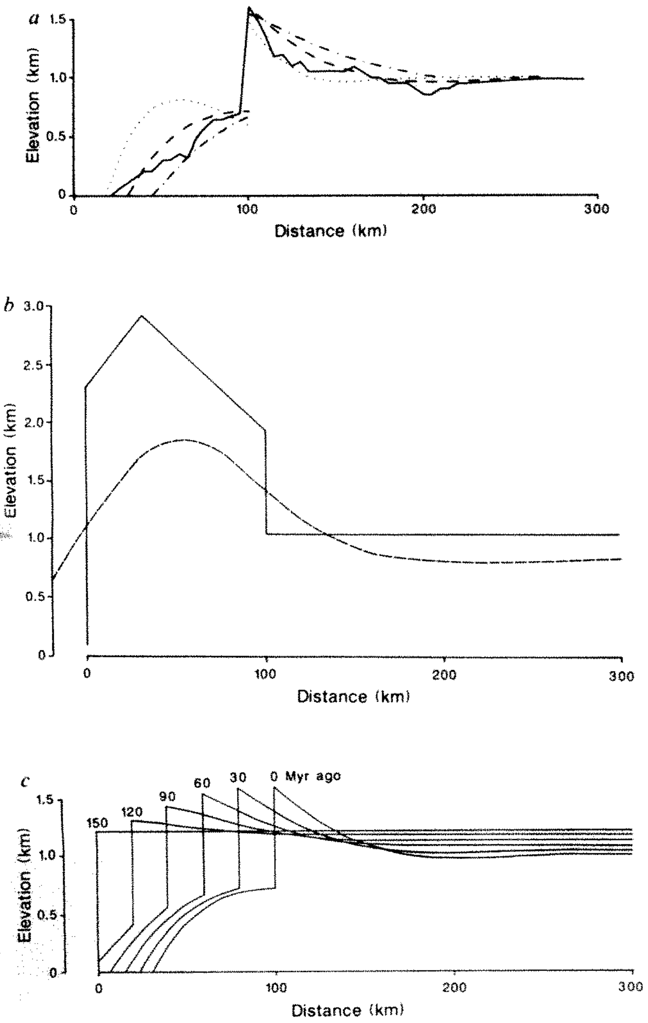

FIG. 2 *a*, Calculated topographic profiles for various T_e values (dotted line, $T_e=7.5$ km; dashed line, $T_e=15.0$ km; dashed-dotted line, $T_e=22.5$ km) compared with a typical topographic profile (solid line) across the western margin of southern Africa. *b*, Total denudation (solid line) and total flexural uplift (dashed line) of the margin. *c*, Evolution of the calculated profiles for $T_e=15$ km. Profile location (X–Y) is indicated on Fig. 1. Zero on horizontal axes indicates location of hinge line. Flexural model parameters were $E=1\times10^{11}$ N m^{-2}, $\nu=0.25$, $g=9.81$ m s^{-2}, $\rho_m=3{,}300$ kg m^{-3}.

We have modelled the denudation of two terrain types characterized by high and low local relief and separated by a moving boundary. The present mean local relief, based on 10-min areas (~18 × 18 km), of the coastal catchments south of the mouth of the Orange River is 456 m, whereas that of the interior Orange catchment is 191 m. Given the linear relationship between mean local relief and denudation rate[18], this indicates that the present mean denudation rate of the coastal catchments is ~2.4 times that of the interior. Given the morphological constraints provided by the present topography and the significantly greater depths of post-rifting denudation along the coast we have assumed that this differential has been maintained since rifting of the Cape Basin (~150 Myr ago)[19]. We have modelled the Great Escarpment as retreating from the hinge line to its present location at a constant rate of 667 m Myr^{-1} (equivalent to a denudation rate of 900 m Myr^{-1}). The size of the coastal catchments has been assumed to have increased linearly from zero at the time of rifting to a present area of 686×10^3 km^3 as the Great Escarpment retreated, whereas the total Atlantic-draining area has been assumed to have remained constant at 1.6×10^6 km^2. Although there is some evidence that the drainage of the interior of southern Africa was not integrated with Atlantic drainage until some time after rifting[2,14], we have adopted the simplest interpretation in view of the uncertainty of the detailed drainage history. In any case the presence of internal drainage in the initial post-rifting phase whould have enhanced the differential denudation that we are modelling. Estimating the mean depth of denudation to be 1,800 m for all Atlantic-draining catchments, the mean denudation rates for the coastal and interior Orange catchments are estimated to be 16.5 m Myr^{-1} and 6.9 m Myr^{-1}, respectively. At the coastal boundary the topography is graded to sea level as the escarpment retreats.

In previous attempts to assess the isostatic response to denudational unloading it has either been assumed that denudation rates are linearly proportional to mean regional elevation[20,21], or that denudation follows a simple diffusion equation in which it is assumed that flux of regolith is proportional to the slope gradient[22]. Neither approach provides a valid basis for modelling denudation rates on high-elevation rifted margins. The relationship between regional elevation and denudation rate does not apply because on high-elevation rifted margins low local relief (low slope and channel gradients) prevails on the high interior inland of the escarpment, whereas high local relief typifies the lower elevation terrain on its oceanward flank. Although the diffusion equation may be applicable to homogeneous, regolith-mantled hillslopes[23] it is not capable of modelling regional-scale topography characterized by marked lithological control.

The precise pre-rifting elevation of southern Africa is unknown; nevertheless, taking the mean elevation of present-day Atlantic-draining catchments of ~930 m and the depth of post-rifting denudation of 1,800 m, the mean pre-rifting elevation of the region of Atlantic-draining catchments can be estimated by reloading the material represented by offshore sediment thickness (d) and allowing for adjustment by Airy isostasy. The pre-rifting elevation will have been higher than present elevation by h, where $h=d(\rho_m-\rho_c)/\rho_m$. For a crustal density ρ_c of 2,800 kg m^{-3} and mantle density ρ_m of 3,300 kg m^{-3} the mean pre-rifting elevation of the Atlantic-draining catchment area is estimated to have been ~1,200 m. In this simple model we have ignored surface uplift associated with the thermo-mechanical evolution of the margin because it would be substantially compensated by sediment loading offshore. To assess specifically the development of the marginal upwarp, the initial topography is assumed to be a block terminating at the rift hinge line.

The isostatic response to denudational unloading has been modelled assuming a thin elastic plate of constant thickness overlying an inviscid fluid according to

$$\frac{\partial^2}{\partial x^2}\left(D\frac{\partial^2 w}{\partial x^2}\right)+(\rho_m-\rho_i)gw=l(x)$$

where w is the vertical flexure, D is the flexural rigidity, ρ_m is the mantle density, ρ_i is the flexure infill density (in this case air), g is the acceleration due to gravity, and $l(x)$ is the denudational load as a function of x (ref. 24). Flexural rigidity is given by

$$D=\frac{ET_e^3}{12(1-\nu^2)}$$

where E is Young's modulus, T_e is the effective elastic thickness of the lithosphere and ν is Poisson's ratio. Vertical flexure was calculated in the wavenumber domain from

$$W(k)=R(k)\cdot L(k)$$

where $R(k)$ is the isostatic response function so that

$$R(k)=\frac{1}{(\rho_m-\rho_i)g+Dk^4}$$

$W(k)$ and $L(k)$ are the Fourier transforms of $w(x)$ and $l(x)$ and k is wavenumber given by $k=2\pi/\lambda$ where λ is the wavelength.

Figure 2*a* shows topographic profiles calculated using different T_e values compared with a typical profile across the western margin of southern Africa (Fig. 1). The elastic thickness of the highly faulted lithosphere below the Cape Basin has been estimated at 5-10 km (ref. 25) whereas that of the cratonic interior is thought to be 64-90 km (ref. 26). Our best fit of 15 km is therefore consistent with this range of values given the intermediate location of the topographic profile between these two types of flexural terrane. In the region of the escarpment our model generates a maximum of ~600 m of surface uplift with respect to the slowly eroding continental interior and closely replicates the characteristic curved profile of the marginal upwarp. Given the spatial variation in total denudation (Fig. 2*b*) (which has also been recorded on other rifted margins[27]), and assuming that compensation to unloading occurs flexurally, the creation of a marginal upwarp is inevitable. Varying the T_e changes the distribution of the upwarped region but the zone of maximum surface uplift remains in approximately the same position. The mismatch between modelled ($T_e=15$) and actual profile on the inland flank of the upwarp is probably attributable to enhanced rates of denudation in this zone associated with back-cutting tributary drainage systems, which have not been incorporated into the model. The evolution of the marginal upwarp as the escarpment retreats is shown in Fig. 2*c*.

The generation of marginal upwarps as a result of differential denudational unloading not only explains their presence along mature rifted margins but also has important geomorphological and sedimentological implications. The presence of marginal upwarps up to 100 Myr or more after rifting may account for the deflection of drainage systems away from many mature rifted margins[2,12,28]. The persistence of such a barrier to sediment transport across high-elevation rifted margins suggests that patterns of offshore deposition are likely to be controlled by a dual drainage system comprising numerous small aggressively eroding coastal catchments and widely spaced outlets of large basins draining continental interiors well into the mature rifted margin stage. Where drainage systems breach a marginal upwarp, the isostatically generated uplift will maintain a significant change in channel gradient (such as the Augrabies Falls on the Orange River). Moreover, the predicted inland migration of the axes of marginal upwarps in response to escarpment retreat has implications for existing denudation and surface uplift chronologies for the Gondwana continents[29]. Further development of our model requires an assessment of the role of denudational unloading along other rifted margins and the more detailed incorporation of apatite fission-track data which, in southwestern Africa at least, indicate significant denudation inland of the present escarpment shortly after rifting during the Early Cretaceous[15]. ☐

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Pyrite formation linked with hydrogen evolution under anaerobic conditions

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THE formation of pyrite (FeS_2), an important factor in determining the global redox balance[1], has recently attracted biological interest as a possible direct source of energy for early life[2–5]. The theory implies that carbon dioxide fixation, in competition with hydrogen formation, can serve as the electron sink for pyrite formation and it seems to be supported by the detection of minute grains of pyrite and iron sulphides inside bacteria[5–8]. Yet it clashes with the conventional assumption that elemental sulphur or a sulphur equivalent (polysulphide or thiosulphate) is the mandatory oxidant for pyrite formation[9,10]. It has been stressed that the reaction $FeS+H_2S\rightarrow FeS_2+H_2$ (with H^+ as the oxidant) has "never been observed ... during several years of experimentation"[10]. Here we report the formation of both pyrite and molecular hydrogen under fastidiously anaerobic conditions in the aqueous system of FeS and H_2S.

Of the geochemical environments in which pyrite can form, two are of particular biological significance: sedimentary systems, in which pyrrhotite ($Fe_{1-x}S$) is extremely rare[11] and in

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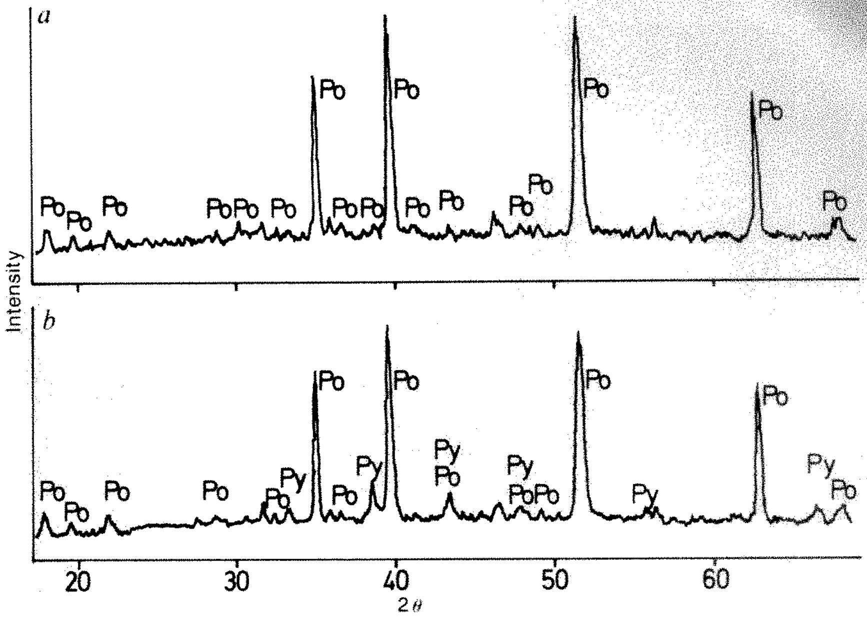

FIG. 1 X-ray diffraction pattern of pyrrhotite (99%; Johnson Matthey). *a*, Starting material. *b*, After incubation at 100 °C for 14 d in aqueous solution in the presence of H_2S. Po, pyrrhotite; Py, pyrite; 2θ, angle of reflection for Co $K\alpha$ radiation.

which pyrite seems to be formed from amorphous FeS[10,12], and hydrothermal systems in which pyrite may be formed not only from amorphous FeS but also from pyrrhotite[11]. We have modelled these by reacting aqueous H_2S at 100 °C for 14 days, under strictly anaerobic and nearly neutral conditions, either with amorphous FeS, precipitated from aqueous $FeSO_4$, or with synthetic (metal basis) pyrrhotite. Our experiments show a linkage between pyrite formation (ascertained by X-ray diffraction) and hydrogen evolution (determined by gas chromatography). Typical results are shown in Table 1 and in Figs 1–3. The pyrrhotite crystals (runs 1, 2) seem to acquire a surface coating of pyrite as indicated by the hollow shells that remain if the pyrrhotite is leached out with HCl (Fig. 2). Amorphous FeS, precipitated in the absence of Fe^{3+} and of sulphur or sulphur equivalents (run 3), seems to produce pyrite in the form of discrete crystals (Fig. 3) as well as some mackinawite (FeS_{1-x}). Hydrogen evolution is also observed with an amorphous FeS precipitated with $Na_2S\cdot 9H_2O$ (containing polysulphide) without removing Fe^{3+} (run 4).

The results may best be represented by the following overall reactions

$$FeS + H_2S \rightarrow Fe(SH)_2$$

$$Fe(SH)_2 \rightarrow FeS_2 + H_2$$

The reaction mechanism may be a concerted four-centre reaction with a simultaneous formation of the covalent bonds of H_2 and S_2^{2-}, but radical or hydride involvement cannot be excluded. The reaction of pyrrhotite seems to be a surface redox reaction with a concomitant rearrangement of the S^{2-}/S_2^{2-} lattice and a diffusion of ferrous ions. The electron transfer may well be promoted by the semiconductor properties of pyrite.

The considerable variety of geochemical environments that give rise to pyrite may indicate a variety of pyrite-forming pathways using different oxidants. By contrast, the conventional assumption that pyrite formation requires not only H_2S (for FeS formation) but also elemental sulphur or polysulphide as the oxidant placed severe restrictions on the possible geochemical explanations of pyrite formation. In particular, it was difficult to explain sedimentary pyrite formation under anaerobic conditions. For example, Boesen and Postma[12] demonstrated that the freshwater Ancylus clay in the Gotland deep of the Baltic Sea (deposited 9,200–7,700 years ago) is presently being sulphidized by the downward diffusion of H_2S from the overlying marine muds. In this process the ferrous ions in the clay are first converted into FeS and subsequently into FeS_2. On this conversion they comment "... unless a hitherto unknown oxidant exists the only explanation seems to be either downward diffusion of

TABLE 1 Products of anaerobic FeS–H_2S systems

	Starting materials		Products after 14 days	
No.	FeS	H_2S (mmol)	H_2 (μmol)	Mineral products
1*a*	pyrrhotite 99%*	2	23 ± 3.5	pyrite
1*b*	(200 mg)	—	0.25	—
2*a*	pyrrhotite 99.99%*	2	18	pyrite
2*b*	(200 mg)	—	0.2	—
3*a*	FeS amorphous, wet†	2	15 ± 4	pyrite + mackinawite
3*b*	(precipitated with H_2S) (2 mmol)	—	0	—
4*a*	FeS amorphous, dried‡	2	40 ± 2.5	pyrite + mackinawite
4*b*	Na_2S (200 mg)	—	0.2	—
5	—	2	0.2	—
6	—	—	0	—

All procedures were carried out under CO_2. The solutions were prepared from doubly distilled water, through which N_2–CO_2 had been bubbled for 2 h. Serum bottles (120 ml) were charged with the suspension of FeS, stoppered and supplied with a N_2–CO_2 atmosphere (80:20, 100 kPa) and then charged with an injection of 2 mmol H_2S gas and adjusted to pH 6.5 with NaOH. The H_2S gas was prepared by adding 50% H_2SO_4 to $Na_2S\cdot 9H_2O$ in an evacuated serum bottle. During incubation for 14 d at 100 °C in a rotary shaker (100 r.p.m.), the serum bottles were kept in anaerobic cylinders with an N_2–CO_2 atmosphere (80:20, 180 kPa). H_2 was determined by gas chromatography (Hewlett Packard 5890). A packed column filled with Molecular Sieve 5A (Supelco) was used (injection temperature, 190 °C; oven temperature, 140 °C; detection temperature, 220 °C; carrier gas, N_2). For runs 1, 3 and 4, the averages and the standard deviations of the H_2 measurements of three repeats of the reaction are given. Run 2 was not repeated. The traces of H_2 in control runs 1*b*, 2*b* and 4*b* are barely above the background (detection limit 0.1 μmol) and may be due to the reaction $2FeS + 2H^+ \rightarrow FeS_2 + Fe^{2+} + H_2$. In control run 5, the trace of H_2 may be due to thermal decomposition ($H_2S \rightleftharpoons H_2 + S$). The solid phase was dried in an anaerobic chamber ($N_2:H_2 = 95:5$) and the mineral composition was analysed by X-ray diffraction.

* Pyrrhotite (99% or 99.99%) (Johnson Matthey) was suspended in 10 ml H_2O. Both pyrrhotites were free of elemental iron as indicated by the lack of hydrogen evolution upon dissolution in concentrated HCl.

† Amorphous wet FeS was precipitated directly in the serum bottles used in the experiment by adding 2 mmol H_2S gas to 10 ml 0.2 M $FeSO_4$ which had previously been freed of Fe^{3+} by treatment with elemental zinc at 60 °C for 2 h.

‡ Amorphous, dried FeS was prepared in an anaerobic chamber by adding $Na_2S\cdot 9H_2O$ (130 g) to 0.6 M $FeSO_4$ that had not been freed of Fe^{3+} filtering the precipitate, washing it with H_2O and drying it under CO_2. The dried precipitate was suspended in 10 ml H_2O.

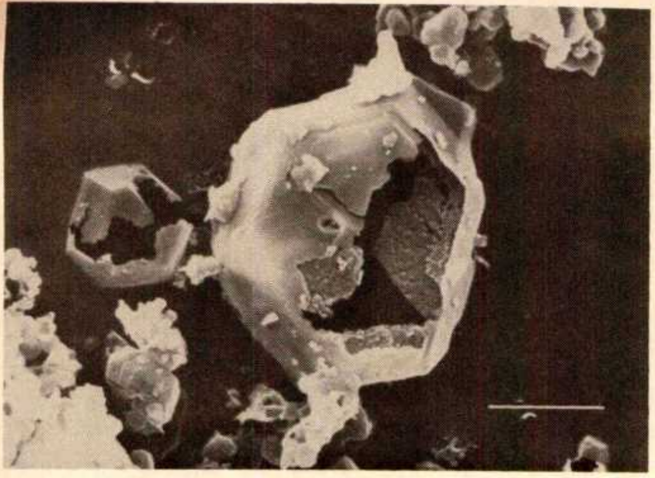

FIG. 2 Scanning electron micrograph of pyrite shells with pyrrhotite pseudomorphy (run 1*a*) after dissolution of pyrrhotite by treatment with 6N HCl. Scale bar, 0.5 μm.

polysulfides or that the FeS/FeS$_2$ distribution reflects a historical change [of the redox conditions]. The latter possibility seems, however, unlikely ....” Our results show that the system FeS–H$_2$S is a powerful reducing agent and that H$^+$ can indeed serve as oxidant for pyrite formation. Thus, the somewhat implausible assumption of a slow diffusion of metastable polysulphides in, for example, the sediments of the Gotland deep is no longer required.

The pyritization of fossils is also a highly variable and complex process. For some of these pyritization processes, our results seem to offer straightforward explanations if we assume: (1) diffusion of dissolved ferrous ions and H$_2$S, at concentrations too small for FeS precipitation (at lower pH, for example) into the site of fossilization; (2) the formation of FeS$_2$ and H$_2$ under the pH conditions of the site of fossilization; (3) the diffusion of H$_2$ out of the site of fossilization. As a variation of this scheme, we may assume diffusion of sulphate ions into the site of fossilization and the formation of hydrogen sulphide by organotrophic sulphate-reducing bacteria at the site of fossilization. In any case, the implausible assumption of a conversion of hydrogen sulphide into elemental sulphur[13] under the reducing conditions at the site of fossilization is no longer necessary.

The production of H$_2$ in nature has previously been attributed to biogenic origins, geothermal exhalations and tropospheric decompositions[14]. Our results establish the system FeS–H$_2$S as an alternative source of hydrogen. This source is ubiquitous, which correlates well with the ubiquitous occurrence of hydrogen-consuming bacteria, such as methanogens, sulphur and sulphate reducers and hydrogen-oxidizing bacteria. Finally, our findings suggest that a functional evolutionary connection might exist between the hydrogen-producing system FeS–H$_2$S and the hydrogen-producing iron–sulphur centres of hydrogenases and nitrogenases. □

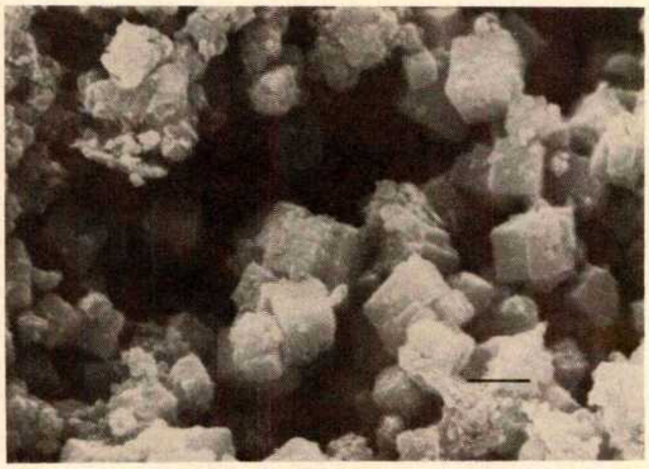

FIG. 3 Scanning electron micrograph of cubic pyrite crystals formed from amorphous FeS (run 4*a*). Scale bar, 1 μm.

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Source-area determination of elephant ivory by isotopic analysis

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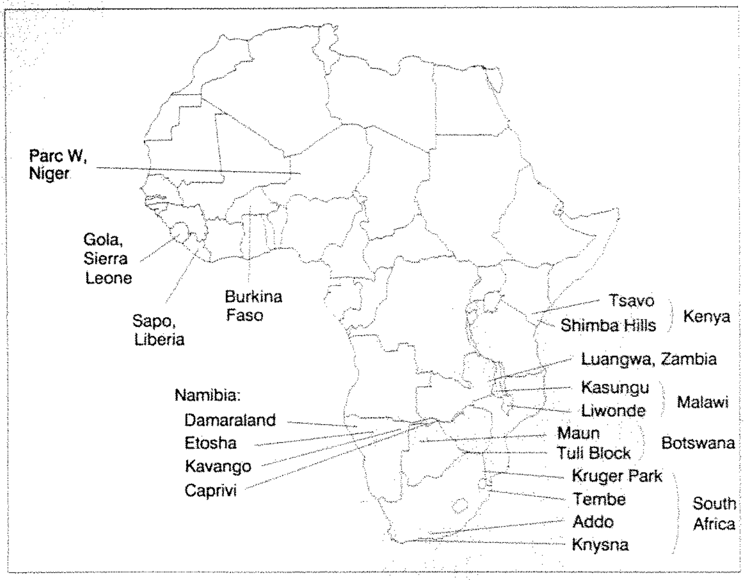
RECENT international efforts to conserve the African elephant *Loxodonta africana* prompted us to seek an appropriate method for determining the area from which individual tusks were derived. Trace element analysis of ivory has indicated the potential of chemical analysis for source identification[1], but recent isotopic studies of African mammals[2–5] suggest another approach. Stable carbon isotope ratios ($^{13}C/^{12}C$) in elephant bone collagen clearly reflect the mixture of C_3 foliage and C_4 grasses in the diet, and are directly proportional to the density of C_3 browse[2]. Furthermore, nitrogen isotope ratios ($^{15}N/^{14}N$) in bone collagen of African mammals are related to rainfall or water stress[3–5]. Strontium isotopes ($^{87}Sr/^{86}Sr$) in bone or ivory can be expected to reflect local geology[6,7]. Here we report on a study of ivory and bone samples from different regions of Africa demonstrating the feasibility of trivariate isotopic analysis to identify the area in which an elephant lived, thus providing a potentially powerful tool for the control of illegal trading in ivory.

For this pilot project, we obtained ivory and/or bone specimens from more than 100 adult elephants, collected by wildlife conservation officers or the police in over 20 identifiable game



◄ FIG. 1 Localities of African game reserves from which elephant samples were obtained for isotopic analysis.

refuge areas in 10 African countries (Fig. 1). Collagen was extracted from the bone or dentine samples and converted to carbon dioxide and nitrogen, according to procedures described elsewhere[2,5,8]. $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios were measured in the VG 602E mass spectrometer of the Archaeometry Laboratory, University of Cape Town; the results are reported as $\delta^{13}C$ and $\delta^{15}N$ values, relative to international standards (Table 1). Carbon and nitrogen isotope ratios obtained from ivory and bone are used interchangeably since x^2 statistical tests indicate no significant difference ($P < 0.05$) between $\delta^{13}C$ values for samples of dentine and bone from the same individuals (elephants and other African mammals[8]). For $^{87}Sr/^{86}Sr$ measurements, strontium nitrate was extracted from ashed samples by standard ion exchange techniques and the isotope ratios determined in the VG 354 thermal ionization mass spectrometer of the Bernard Price Institute for Geophysical Research, University of the Witwatersrand.

$\delta^{13}C$ and $\delta^{15}N$ values obtained for elephants from different regions of Africa (Fig. 2) are patterned as predicted. In woodland savannahs, elephants eat primarily C_3 plants (plants carrying out carbon dioxide fixation through the Calvin cycle) and have collagen $\delta^{13}C$ values near −21.5‰ (such as Parc W in Niger, Shimba Hills in Kenya, and Skukuza in Kruger Park, South Africa). This value has been well documented for C_3 plant consumers[9,10]. In dense forests (Sapo, Gola, Knysna), where isotopically light CO_2 is recycled under the forest canopy[11,13], $\delta^{13}C$ values are more negative than −22‰; the denser the forest, the more negative the $\delta^{13}C$ value. This phenomenon, by itself, tends to distinguish elephants from the forested tropics. Values more positive than −21.5‰ indicate C_4 grass (plants carrying out carbon dioxide fixation through the C_4 pathway) in the diet as shown, for example, by samples from Tsavo East. An exception to this pattern is Addo, South Africa, where a substantial proportion of the browse consists of CAM plants (those plants in which carbon dioxide fixation proceeds initially through crassulacean acid metabolism) (including *Portulacaria afra*, mean $\delta^{13}C$ value −17,7‰ (ref. 2)). In the case of nitrogen isotope ratios, samples from arid zones (northwestern Namibia, Addo and Tsavo East) have high $\delta^{15}N$ values, as expected for areas where mean annual rainfall is <400 mm. The CAM plants of Addo are particularly enriched in ^{15}N (ref. 14) and contribute to high $\delta^{15}N$ values for Addo elephants.

A correlation between $\delta^{13}C$ and $\delta^{15}N$ values for elephant samples from areas in southern Africa (Fig. 2) is a reflection of relationships between climate and vegetation. Low $\delta^{15}N$ values are obtained for samples from areas with relatively high rainfall and a substantial degree of C_3 (bush/tree) vegetation;

FIG. 2 Distribution of $\delta^{13}C$ and $\delta^{15}N$ values obtained from elephant ivory and bone samples from *a*, West Africa (Liberia, Sierra Leone, Burkina Faso), East Africa (Tsavo, Shimba Hills), and south-central regions (Malawi and Zambia), *b*, South Africa (Kruger Park, Knysna, Addo), *c*, Namibia and Botswana. $\delta^{13}C$ and $\delta^{15}N$ values serve to distinguish source areas in most cases; strontium isotope ratios (Table 1) serve to distinguish elephants from reserves such as Luangwa and Liwonde that have overlapping $\delta^{13}C$ and $\delta^{15}N$ distributions.

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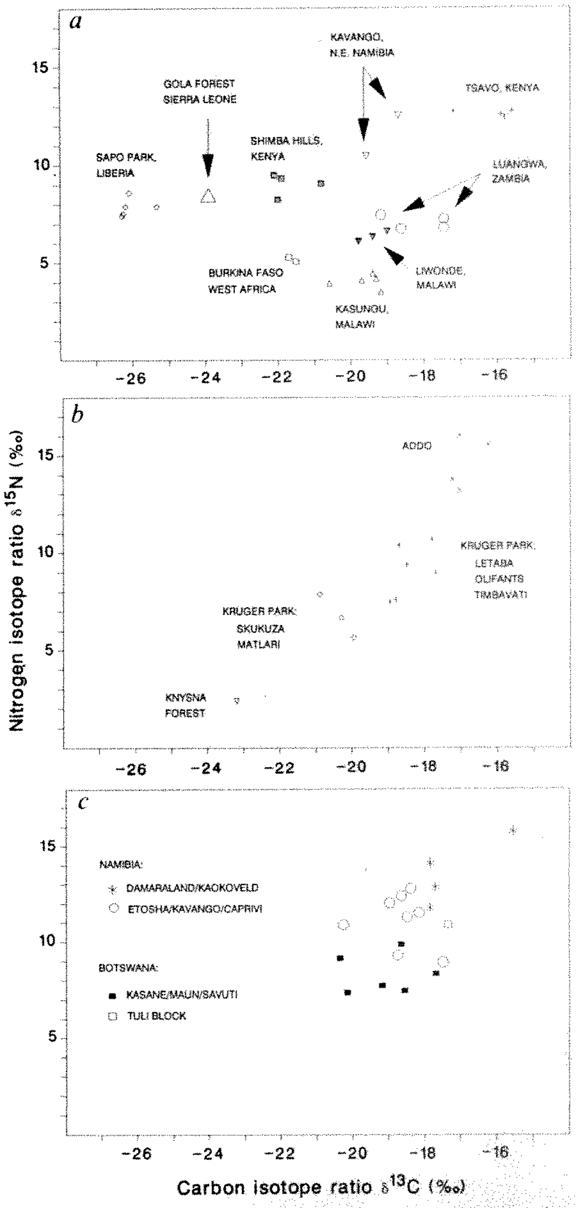

TABLE 1 Isotopic analysis of collagen from elephant bone and dentine

Country	Region	$\delta^{13}C$	s.d.	n	$\delta^{15}N$	s.d.	n	$^{87}Sr/^{86}Sr$	s.d.	n
Liberia	Sapo	−26.80	0.75	12	7.77	0.46	5	0.71191	—	2
Sierra Leone	Gola forest	−24.00	—	1	8.80	—	1	0.73314	—	1
Burkina Faso		−21.60	—	2	5.20	—	2	0.71493	—	2
Niger	Parc W	−20.83	0.36	5	5.70	—	2	—	—	—
Kenya	Shimba Hills	−21.70	0.52	4	9.04	0.48	4	0.71238	0.00031	3
Kenya	Tsavo	−16.10	0.64	4	12.58	0.13	4	0.71000	—	2
Malawi	Kasungu	−19.62	0.47	6	4.03	0.32	5	0.72683	0.00850	4
Malawi	Liwonde	−19.51	0.32	3	6.40	0.16	3	0.71101	0.00084	3
Zambia	Luangwa Valley	−18.16	0.70	4	6.78	0.52	4	0.72155	0.00168	3
Botswana	(Kasane, Maun, Savuti)	−19.22	0.9	6	8.66	0.68	6	0.72126	0.00071	3
Botswana	Tuli Block	−17.20	—	1	10.80	—	1	0.70649	—	1
Namibia	Damaraland, Kaokoveld	−17.15	1.01	4	13.38	1.44	4	0.72426	0.00068	3
Namibia	Etosha	−18.02	0.33	3	10.55	1.81	3	0.71646	0.00187	5
Namibia	Kavango/Caprivi	−18.85	0.51	5	11.12	1.15	5	0.71928	0.00109	5
South Africa	Kruger Park (east)									
	Olifants	−18.34	—	2	8.25	—	2	0.70799	—	1
	Letaba	−18.60	—	2	9.90	—	2	0.71178	—	2
	Timbavati	−18.25	—	2	8.80	—	2	0.71526	—	1
	Total	−18.40	0.52	6	8.98	1.11	6	0.71171	0.00264	4
South Africa	Kruger Park (west)									
	Matlari	−20.53	0.45	4	7.29	—	2	—	—	—
	Skukuza	−21.16	0.96	7	5.70	—	1	0.72406	—	1
	Total	−20.94	0.87	11	6.77	0.90	3	0.72406	—	1
South Africa	Tembe (Kwazulu)	−19.47	1.14	6	8.88	0.33	4	0.71426	—	2
South Africa	Addo	−17.03	0.37	8	14.64	1.19	6	0.71154	—	2
South Africa	Knysna	−23.20	—	1	2.40	—	1	—	—	—

Mean values and standard deviations obtained for $\delta^{13}C$, $\delta^{15}N$ and $^{87}Sr/^{86}Sr$ from samples of elephant ivory and bone from African game refuges; most if not all samples were obtained from adult elephants from different herds. Carbon and nitrogen isotope ratios are reported in the δ notation as per thousand (‰) relative to the PDB standard and air N_2, respectively. Precision (including instrument error) is $<0.1‰$ for $\delta^{13}C$ and $<0.5‰$ for $\delta^{15}N$ values. Strontium isotope ratios ($^{87}Sr/^{86}Sr$) may be compared with a mean value of 0.710217 ± 0.000030 (precision of 0.004%) for NBS-987 standard.

conversely, high $\delta^{15}N$ values are obtained for samples from areas with lower rainfall and relatively more C_4 vegetation, notably in the desert regions of Damaraland and Kaokoveld in north-western Namibia.

The numbers of measurements obtained from each refuge in this pilot study do not lend themselves to detailed statistical analysis, but $\delta^{13}C$ and $\delta^{15}N$ values are clearly clustered for each region (Fig. 2). For areas where $n \geq 5$, standard deviations are small, between 0.36 and 1.14 for $\delta^{13}C$, and between 0.32 and 1.19 for $\delta^{15}N$. Intra-area variation is comparable to intratusk variability, as determined for a tusk from northern Namibia (mean $\delta^{13}C = -17.6 \pm 0.8‰$, $n = 7$; mean $\delta^{15}N = 8.1 \pm 0.5‰$, $n = 7$). Intratusk variability is attributable to seasonal variation in the diet and/or seasonal movements, both of which are particularly marked in this desert region. Statistical t-tests indicate significant differences ($P < 0.05$) between mean $\delta^{13}C$ values for elephant samples from Tsavo East and Shimba Hills in Kenya, only 150 km apart, and between mean $\delta^{15}N$ values for samples from the same reserves; similarly, mean $\delta^{13}C$ and $\delta^{15}N$ ratios for samples from eastern and western sectors of the Kruger Park (Fig. 2*b*) are significantly different ($P < 0.05$).

Overlap in the distributions of carbon and nitrogen isotope ratios can be expected when dealing with elephants from similar environments. In such cases, strontium isotopes can be used to make distinctions, as $^{87}Sr/^{86}Sr$ ratios determined from tusk or bone reflect the age and average Rb/Sr ratios of the parent rocks in a particular region[6,7]. Young volcanic soils (in the Rift Valley for example) are particularly noticeable in this regard as they have ratios similar to 0.70906, the value for ocean water. Samples from Liwonde (Malawi) and Luangwa (Zambia) have overlapping $\delta^{13}C$ and $\delta^{15}N$ distributions (Fig. 2*a*), but they have clear differences in $^{87}Sr/^{86}Sr$ ratios (Table 1). Similarly, $\delta^{13}C$ and $\delta^{15}N$ values for samples from Kruger Park (Fig. 2*b*) overlap with those obtained for elephants from similar environments in Botswana and northern Namibia (Fig. 2*c*), but strontium isotope ratios differ (Table 1) because of geological history. In general, samples from areas with soils which developed on old granitic rocks (shield areas) have $^{87}Sr/^{86}Sr$ ratios that are >0.715, whereas those from areas with rocks of basaltic composition have lower ratios (for example, Tuli Block in Botswana).

We conclude that trivariate isotopic analysis provides a potential means of identifying source areas of ivory on a wide geographic scale. Practical application of this method will require a more comprehensive index of ivory (or bone) isotope signatures for all regions where elephants still exist in Africa. Such a database could provide the foundation for international control of the ivory trade and hence for the conservation of the elephant and its habitat. ☐

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Isotope fingerprints in elephant bone and ivory

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THE isotopic composition of carbon and nitrogen as well as of strontium in animal bone is related to the environment in which the animal lived[1-6]. It can be assumed that this is also the case for lead isotopes. In theory, therefore, we have a way of pinpointing the origin of elephant ivory, which may be of value in conservation. Here we report that by analysing the isotope ratios of these elements, a clear distinction between several different populations of the African elephant can be made.

We used the isotope signatures to characterize elephant populations in different habitats in southern Africa (Fig. 1). In most cases bone from the jaws or ribs was used, but some samples of ivory were also included. The specimens came from the Knysna Forest on the south coast of the Cape Province, the Addo Elephant National Park in the southwest Cape, different areas of the Kruger National Park, East Transvaal, and the northern Namib Desert in Namibia. Three single samples from areas to the east of the latter group, namely East Kaokoveld, the Etosha Game Reserve and Caprivi, complete the list.

The factors expected to determine the isotope ratios of the various elements in the skeletons of elephants are briefly discussed below. In most parts of Africa the $^{13}C/^{12}C$ ratio in animal tissue is determined primarily by the amount of (C_4) grass ingested by the animal[1] and, thus, in the case of mixed feeders such as elephants, which both graze and browse, is to a degree dependent on the environment[2]. The $^{15}N/^{14}N$ ratio in bone collagen of ungulates, is, again, higher in more arid habitats[3,4]. This may be ascribed, at least in part, to the higher ^{15}N levels of the local vegetation[5], and for the rest it has been proposed to be the effect of drought stress on the protein metabolism of the animals themselves[6]. The carbon and nitrogen isotopes, even when taken together, do not, however, completely characterize the various elephant populations of southern Africa[7]. Only by adding a further independent isotope signature can this be achieved.

The $^{87}Sr/^{86}Sr$ ratio in animal bone is determined by that of the strontium in the animal's plant diet, which is determined by the ratios in the soil and water of the area[8,9]. In most cases these ratios reflect those of the underlying rocks. ^{87}Sr is produced by the slow radioactive decay of ^{87}Rb, so that its abundance depends on the rubidium content and the age of the rock suites in the substrata of the area. Consequently, the higher the Rb/Sr ratio and the older the rock, the higher the $^{87}Sr/^{86}Sr$ ratio will be.

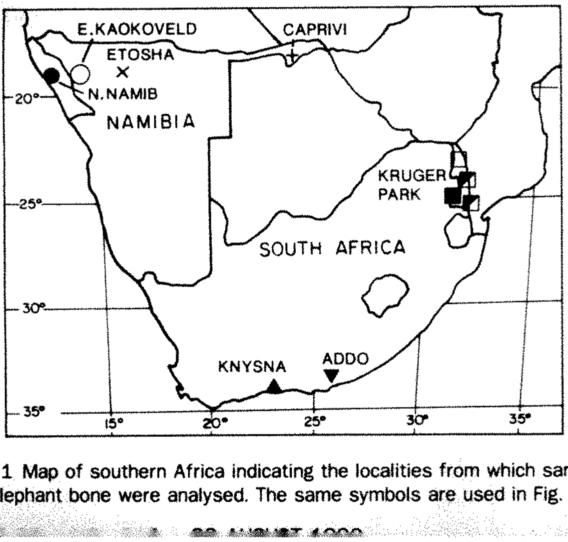

FIG. 1 Map of southern Africa indicating the localities from which samples of elephant bone were analysed. The same symbols are used in Fig. 2.

The lead isotopes, which are decay products of uranium and thorium, have not been used previously on bone material in the same way as have the strontium isotopes. Lead substitutes for calcium in a similar manner to strontium and the lead isotope ratios should thus also be characteristic of the underlying rocks.

Standard procedures were used to prepare samples for isotope analysis in suitable mass spectrometers. The results are listed in Table 1. ^{13}C analysis only distinguishes the Knysna specimens from the rest, whereas ^{15}N clearly distinguishes Knysna, Kruger Park and Addo samples, with the Namibian specimens falling between and overlapping the latter two groups. The ^{87}Sr, again, distinguishes the Namibian samples from the rest, the higher ^{87}Sr values being associated with areas on older, more felsic substrata (see Table 1). It is also noteworthy that the specimens from the Archaean granitic area in the far southwest of the Kruger Park have distinctly higher ^{87}Sr values than do those from the younger Mesozoic basalts in the far northeast, as is to be expected. The three lead isotopes show close correlation with each other, but do not effectively distinguish the different regions from each other, although there is a tendency for the isotope ratios in Addo and most of the Kruger Park specimens to be lower than those in Knysna and the Namib.

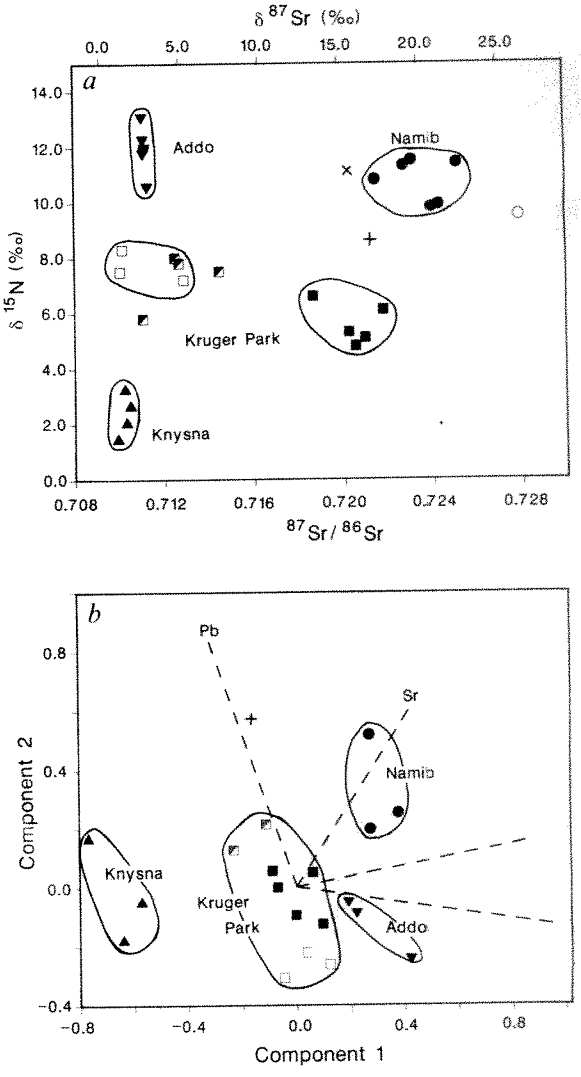

FIG. 2 *a*, Plot of the relative ^{15}N and ^{87}Sr contents of elephant bone, showing the complete separation of the different elephant populations from Knysna, Addo, Kruger Park and the N. Namib Desert (Table 1). The specimens belonging to specific populations are encircled. *b*, Plot of the first two principal components of the multivariate statistical analysis of the specimens for which measurements of the isotopic composition of carbon, nitrogen, strontium and lead were made.

TABLE 1 Isotope ratio measurements of elephant bone and tusk from localities in southern Africa*

Sample no.	Locality	Main rock substrate	Material	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$^{87}Sr/^{86}Sr$	$\delta^{87}Sr$ (‰)	$^{206}Pb/^{204}Pb$	$^{207}Pb/^{204}Pb$	$^{208}Pb/^{204}Pb$
B730	Knysna Forest, S Cape Prov	Palaeozoic sandstones, Table Mountain Group	Bone	−23.6	+3.0	0.710609	+2.0	17.295	15.561	37.435
B765			Bone	−23.8	+1.2	0.710281	+1.5	16.919	15.509	36.851
C4099			Tusk	−24.5	+2.4	0.710845	+2.3			
C4100			Bone	−24.7	+1.8	0.710676	+2.1	18.039	15.724	38.905
			Average	−24.2	+2.1	0.710603	+2.0	17.417	15.598	37.730
			s.d.	±0.5	±0.8	±0.000236	±0.3	±0.569	±0.112	±1.058
B733	Addo Elephant Park, SE Cape Prov.	Mesozoic marine seds, Uitenhage Group	Bone	−19.2	+12.0	0.711482	+3.2			
B734			Bone	−18.8	+11.7	0.711562	+3.3			
B735			Bone	−17.7	+10.3	0.711656	+3.5	17.078	15.502	36.859
B738			Bone	−17.1	+12.8	0.711442	+3.2	16.476	15.458	36.259
B739			Bone	−18.8	+11.6	0.711546	+3.3	17.118	15.538	37.165
B741			Bone	−17.5	+11.5	0.711484	+3.2			
			Average	−18.2	+11.7	0.711529	+3.3	16.891	15.499	36.761
			s.d.	±0.9	±0.8	±0.000077	±0.1	±0.360	±0.040	±0.461
	Kruger Park, E. Tvl*									
B770.1	Far SW. (Skukuza to Malelane	Archaean granitoid gneisses	Bone	−18.9	+6.3	0.719014	+13.8	16.430	15.440	36.131
B770.3			Bone	−19.2	+5.8	0.720617	+16.1	16.804	15.698	36.821
B770.4			Bone	−20.1	+4.5	0.720915	+16.5	16.932	15.649	36.904
B770.5			Bone	−19.8	+4.8	0.721340	+17.1	16.363	15.419	36.037
B770.0			Bone	−20.3	+5.0	0.722530	+18.8	16.725	15.448	36.482
			Average	−19.7	+5.3	0.720883	+16.5	16.651	15.531	36.475
			s.d.	±0.6	±0.7	±0.001273	±1.8	±0.244	±0.132	±0.392
B773.1	Far NE (S of Pafuri)	Mesozoic basalts, Karoo Seq.	Bone	−20.0	+7.2	0.710417	+1.7	16.396	15.460	36.130
B773.2			Bone	−18.0	+8.0	0.710528	+1.9	16.612	15.463	36.336
B773.4			Bone	−19.0	+6.9	0.713263	+5.7	16.526	15.478	36.259
			Average	−19.0	+7.4	0.711403	+3.1	16.511	15.467	36.242
			s.d.	±1.0	±0.7	±0.001612	±2.3	±0.109	±0.010	±0.104
C4069	Far SE (Crocodile Bridge)	Transitional	Tusk	−18.6	+7.2	0.714848	+8.0			
C4068			Bone	−20.0	+5.5	0.711428	+3.1	17.924	15.647	38.552
C4050	Mid S (Tshokwane)	Transitional	Tusk	−17.2	+7.7	0.712864	+5.2			
C4051			Bone	−19.7	+7.5	0.713054	+5.4	18.040	15.728	38.911
	Namibia									
B846	N. Namib Desert	Mid-Late Proterozoic gneisses and schists	Bone	−18.6	+9.5	0.724338	+21.3	17.042	15.529	37.139
B847			Bone	−18.7	+11.1	0.725462	+22.9	18.012	15.718	38.869
B848			Bone	−17.8	+11.2	0.723448	+20.1	17.280	15.548	37.215
B849			Bone	−18.0	+9.6	0.724681	+21.8			
B851			Bone	−18.7	+10.5	0.721813	+17.8			
B853			Bone	−17.9	+11.0	0.723087	+19.6			
			Average	−18.3	+10.5	0.723805	+20.6	17.445	15.598	37.741
			s.d.	±0.4	±0.8	±0.001297	±1.8	±0.506	±0.104	±0.978
B857	E Kaokoveld	Mid Proterozoic granitoid gneisses	Bone	−16.4	+9.2	0.728249	+26.9			
B316	Etosha Game Reserve	Cenozoic sands, Kalahari Group over	Bone	−17.9	+10.8	0.720615	+16.1			
B112	Caprivi	Late Proterozoic meta-seds	Bone	−21.7	+8.2	0.721590	+17.5	18.485	15.655	38.298

* L$\delta^{13}C$, Carbon isotope ratios expressed relative to the PDB reference standard; $\delta^{15}N$, nitrogen isotope ratios relative to atmospheric nitrogen; $\delta^{87}Sr$, strontium isotope ratios relative to average modern-day sea water (0.70920). The reproducibility of the measurements (1 s.d.) is better than 0.4‰ for carbon and nitrogen, 0.1‰ for strontium, and 0.1%, 0.1% and 0.2% for the three lead isotope ratios, respectively. Replicates of NBS SRM 987 strontium carbonate gave an isotope ratio of 0.710275±16 (1 s.d., $n=10$). Mass fractionation corrections for the lead ratios are based on replicates of NBS SRM 981.

† The four groups represent four different populations in the Park. The specimens from the Far SW are from at least three different herds; those from the Far NE are from two separate herds.

For the various elephant populations considered here, complete distinction, without any overlap, is achieved by plotting ^{15}N against ^{87}Sr (Fig. 2*a*).

As further populations from other parts of Africa are added, the characterization based on these two isotopes alone is bound to become less distinct, and multivariate analysis, using all the available isotopes, would be more appropriate. To illustrate this approach, isotope data for the 20 specimens for which lead isotope measurements are also available (using the $^{206}Pb/^{204}Pb$ ratio as representative of the three lead isotopes) have been analysed using multivariate statistics, in the form of combined R- and Q-mode components analysis[10], based on a correlation similarity matrix. Ninety-eight per cent of the total variance in the data set is explained by three components which are essentially dominated by the isotope ratios of C and N (component 1), Pb and Sr (component 2) and Sr (component 3). Distinction of the four populations is quite clear on a plot of component 1 against component 2 (Fig. 2*b*), although plots of the other components could be more effective in distinguishing separate localities within a region.

Most of our analyses were of bone, but there is no reason why ivory cannot also be used. The only difference would be that a sample of ivory taken from the base of the tusk reflects the food intake during the last year or so of the life of the animal, whereas the bone is integrated over most of its lifetime. To check the validity of using ivory, three pairs of bone and ivory from the same animal were analysed (see Table 1). In all three cases the ivory values fall into the same grouping in Fig. 2*a* as do those of the bone. It can, therefore, be concluded with confidence that the combined analysis of ^{15}N and ^{87}Sr in tusks can provide a useful tool for establishing their origin. More distinctive fingerprinting can be achieved by adding other isotope signatures, notably those of lead, carbon and, possibly, sulphur. There would have to be a more complete survey of elephant habitats in the rest of Africa before the technique can be applied on a wide scale. In principle, however these isotope fingerprints in ivory can make a positive contribution towards monitoring the products of the endangered African elephant. ☐

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Maintenance of B-cell memory by long-lived cells generated from proliferating precursors

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A BASIC feature of T-cell dependent antibody responses is the generation of memory: on a second contact with an antigen a secondary response is produced in which somatically mutated antibodies with increased affinity are synthesized[1]. Memory can persist for long periods of time. This has classically been ascribed to the generation of long-lived memory B cells[2,3]. However, it is also possible that persisting antigen, on which memory may depend[4], maintains a population of cycling memory cells under continuous selection[5] or continuously recruits newly generated B cells into the memory B-cell compartment[6]. To discriminate between these mechanisms we have now directly analysed the proliferative activity in the memory B-cell compartment of the mouse by measuring bromodeoxyuridine incorporation *in vivo*. We show that after an initial phase of extensive proliferation after primary immunization, memory cells can persist in the organism for extended periods of time in the absence of cell division.

In accordance with the work of Hayakawa *et al.*[7], we raised specific memory B cells in mice by immunization with the highly fluorescent protein phycoerythrin (PE) and characterized them as PE-binding cells that express the B-cell marker B220 and low levels of surface immunoglobulin differing in class from IgM and IgD as expressed by naive B cells. Using magnetic cell sorting and flow cytometry in combination, we analysed these cells directly for their proliferative activity *in vivo*. The outline of the experiment is given in Fig. 1. At various times after immunization, bromodeoxyuridine (BrdU; 1 mg ml^{-1}) was added to the drinking water of the animals, for various periods of time. Dividing lymphoid cells incorporate BrdU into their DNA under these conditions, and the proliferative behaviour of BrdU-labelled B lineage cells is not different from that of unlabelled cells[8,9]. At the time of analysis, spleen cells were isolated, enriched for IgM$^-$IgD$^-$ B cells on a magnetic cell sorter (MACS)[10], and stained with PE and an anti-B220 antibody. The cells could then be processed in either of two ways: flow cytometric analysis and cell sorting allowed quantitation and functional and morphological analysis of PE binding memory cells; alternatively, the cells were fixed, stained with an anti-BrdU antibody and analysed for BrdU incorporation by three-colour flow cytometry.

PE-binding, B220-positive (B220$^+$PE$^+$) cells were readily detectable among the IgM$^-$IgD$^-$ spleen cells of PE-immunized mice (see Fig. 2*a*). When these cells were examined under the fluorescence microscope, they turned out to be small lymphocytes, negative for IgM and IgG1 in the cytoplasm. To confirm that these cells are functionally active memory cells, we tested them for their ability to produce a secondary antibody response *in vitro*, as described earlier[7]. For this purpose, PE-binding (B220$^+$PE$^+$) and nonbinding B cells (B220$^+$PE$^-$) isolated from the spleens of mice immunized with PE 7 or 12 weeks earlier were cultured in the presence or absence of helper T cells (CD4$^+$) from PE-immunized mice (Table 1). After 14 days the culture fluids were analysed for the presence of PE-specific IgG1 antibodies. As few as 250 B220$^+$PE$^+$ cells, but not B220$^+$PE$^-$ cells produce a strong IgG1 anti-PE-response when (and only when) cultured together with PE-specific helper T cells (Table 1). This confirms that in the PE-primed animals PE-specific memory B cells are located in the B220$^+$PE$^+$ population and that the antibody response measured is not due to plasma cell contamination.

To determine the proliferative activity of these memory cells, the cells were analysed for BrdU incorporation *in vivo*. Feeding of BrdU to the mice was started either 1 day, 4 weeks, 10 weeks or 20 weeks after priming, and stopped 1, 3, 5 or 7 weeks later; the spleen cells of the animals were processed as shown in Fig. 1. Representative staining patterns are shown in Fig. 2. Plotted is either PE against anti-B220 (Fig. 2*a*) or PE against anti-BrdU-staining (Fig. 2*b*). To determine the ratio of labelled versus unlabelled cells among the memory cells, we gated on B220$^+$PE$^+$ cells (Fig. 2*a*), collected 1,000–5,000 cells in this fraction, analysed the BrdU-staining profile (Fig. 2*c–f*) and calculated the percentage of BrdU-labelled cells in this population.

Almost all of the B220$^+$PE$^+$ cells (92%) divide within the first five weeks after priming (Fig. 3*a*). That the values do not reach 100% could indicate that this population contains cells that do not divide at all. More likely, this represents a problem in the evaluation of the staining data, as negative and positive populations were not always fully separated. When BrdU-treatment is

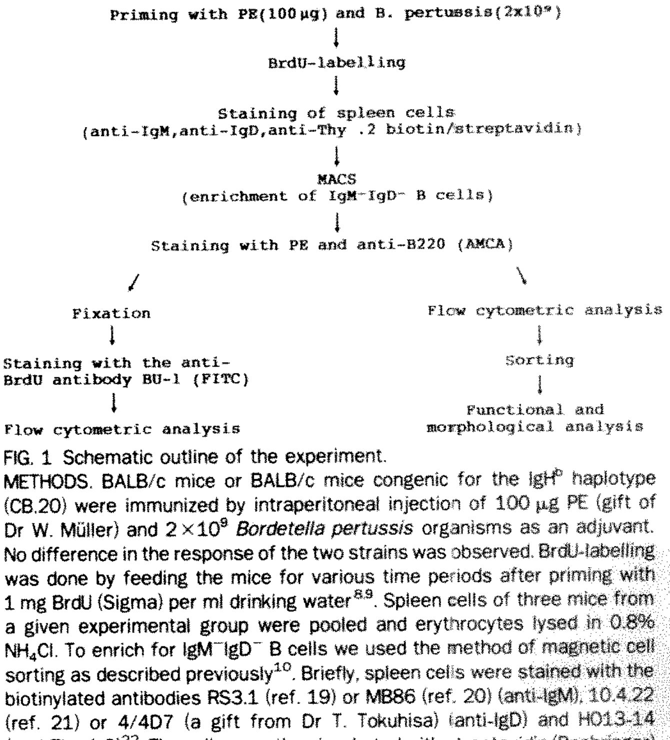
Priming with PE(100 µg) and B. pertussis(2x10⁹)
BrdU-labelling
Staining of spleen cells
(anti-IgM,anti-IgD,anti-Thy .2 biotin/streptavidin)
MACS
(enrichment of IgM⁻IgD⁻ B cells)
Staining with PE and anti-B220 (AMCA)
Fixation
Staining with the anti-BrdU antibody BU-1 (FITC)
Flow cytometric analysis
Flow cytometric analysis
Sorting
Functional and morphological analysis

FIG. 1 Schematic outline of the experiment.

METHODS. BALB/c mice or BALB/c mice congenic for the IgH^b haplotype (CB.20) were immunized by intraperitoneal injection of 100 µg PE (gift of Dr W. Müller) and 2×10^9 *Bordetella pertussis* organisms as an adjuvant. No difference in the response of the two strains was observed. BrdU-labelling was done by feeding the mice for various time periods after priming with 1 mg BrdU (Sigma) per ml drinking water[8,9]. Spleen cells of three mice from a given experimental group were pooled and erythrocytes lysed in 0.8% NH_4Cl. To enrich for IgM$^-$IgD$^-$ B cells we used the method of magnetic cell sorting as described previously[10]. Briefly, spleen cells were stained with the biotinylated antibodies RS3.1 (ref. 19) or MB86 (ref. 20) (anti-IgM), 10.4.22 (ref. 21) or 4/4D7 (a gift from Dr T. Tokuhisa) (anti-IgD) and HO13-14 (anti-Thy-1.2)[22]. The cells were then incubated with streptavidin (Boehringer) and, in a third step, with superparamagnetic biotinylated microparticles. Eight to eighteen percent of the spleen cell population remained unlabelled by this procedure and were enriched to 85–95% using a MACS[10]. The recovery was 70–80%. The enriched cells were stained with PE (10 µg per 5×10^7 cells) and with the anti-B220 antibody RA3.6B2 (ref. 23) coupled to AMCA (Amino-methyl-coumarin-acetic acid)[24] (10 µg per 5×10^7 cells). Cells were fixed in 70% ethanol, washed in PBS and stained with the anti-BrdU antibody BU-1 (refs 25, 26) (culture supernatant; 30 µg per 2×10^7 cells) (kindly provided by Dr N. Gonchoroff) and with an anti-IgG2a^a antibody (Ig(1a)8.3)[21] coupled to fluorescein-isothiocyanate (FITC; 10 µg per 5×10^7 cells). Using this anti-BrdU antibody it is not necessary to denature the cellular DNA by acid or base treatment, which would destroy PE-staining of the cells.

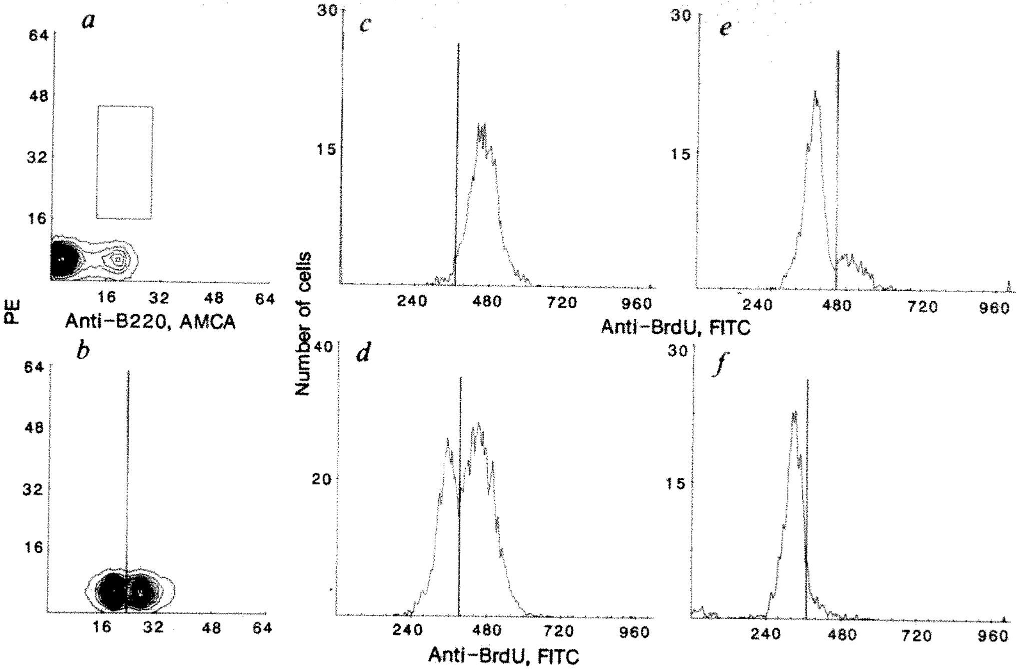

FIG. 2 BrdU-analysis of memory B cells. Shown are representative staining patterns for B220 surface expression and PE-binding (*a*), or BrdU-incorporation and PE-binding (*b*) of spleen cells of PE-immunized mice after enrichment of IgM^-IgD^- B cells. After gating on $B220^+PE^+$ cells (see gate in Fig. 2*a*), between 1,000 and 5,000 of these cells were collected and the BrdU-staining pattern analysed (*c*-*f*). Feeding of BrdU was started either 1 day (*b*, *c*), 4 weeks (*d*), 10 weeks (*e*) or 20 weeks (*f*) after priming. Feeding was stopped after either 19 days (*f*), 34 days (*b*, *c* and *e*) or 48 days (*d*) of BrdU-labelling. Gates were set between positive and negative populations and the percentage of BrdU-labelled versus unlabelled cells calculated according to this gate (see Fig. 3*a*).

METHODS. Enrichment and staining of spleen cells was done as described in Fig. 1. Fluorescence staining was analysed on a FACS Star Plus using three-colour immunofluorescence detection. List mode data were stored in a MicroVax and analysed using Consort 40, version 5.2 (Becton Dickinson). Only cells falling within normal lymphocyte values for both forward and orthogonal scatter are included[9]. Cells with more than 16–25 units of PE-staining intensity were considered as PE-bright (PE^+) (see *a*).

started four weeks after priming and continued for 18 or 33 days, 36% or 62% of the memory B cells are labelled, respectively. Prolongation of the BrdU-treatment to 48 days does not result in a significantly higher percentage of labelled cells in the latter population (64%; Fig. 3*a*). In contrast, when BrdU-treatment is started 10 weeks after immunization, 82–88% of the memory B cells do not divide within 5 or 7 weeks. A similar situation is encountered 20–23 weeks after priming. The low degree of cell proliferation 10–20 weeks after priming indicates either some recruitment of naive cells into the memory B-cell compartment, or proliferation of some memory B-cell clones still at that time. However, the data clearly show that 9–10 weeks after immunization and later, most of the memory cells do not divide and are not recruited from newly generated B cells over extended periods of time (≥7 weeks). The number of $B220^+PE^+$ cells in the spleens of the immunized animals—of the order of 0.01% of the total spleen cell population, a little below the 0.02–0.05% reported by Hayakawa *et al.*[7]—remain essentially constant for at least 200 days, from around 12 days after priming (Fig. 3*b*). This accords with the observation (K. Hayakawa, personal communication) that functionally active, PE-specific memory B cells persist in the animals for at least 17 months after priming. We thus conclude that after an initial period of extensive proliferation and/or recruitment from newly generated cells, memory B cells convert into a population of long-lived cells.

We consider it likely that the incorporation of BrdU seen in the memory compartment in the first weeks after immunization mainly reflects antigen-driven proliferation of precursor B cells, from which somatic mutants expressing antibodies with high affinity for the antigen are selected[1]. It has been suggested[11,12] that this process of proliferation and selection takes place in the germinal centres, which form after primary, T cell-dependent immunization in the peripheral lymphoid organs. The duration of this early, T cell-dependent[13,14] phase of memory generation may depend on the nature of the primary antigenic stimulus. The present data indicate that memory cells generated in this process are long-lived cells that can persist in the organism for a good part of its lifetime. This is in accord with recent data demonstrating that the persistence of memory B cells requires little, if any, T-cell help[14].

These experiments do not address the question of whether

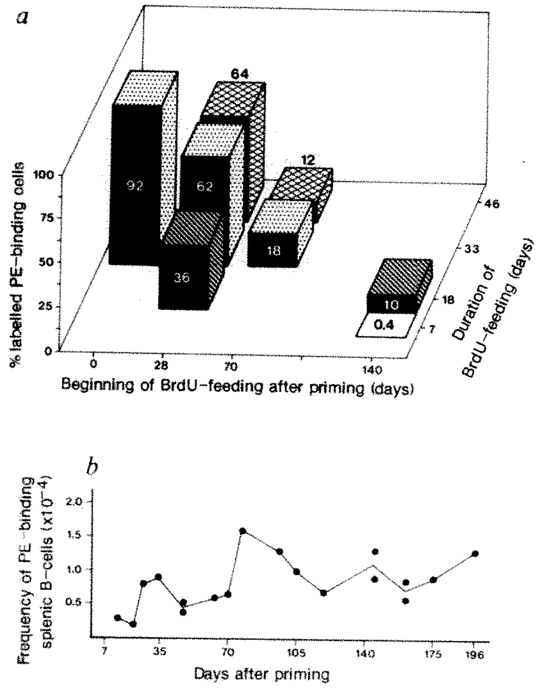

FIG. 3 Summary of the proliferative activity (*a*) and the frequency (*b*) of memory B cells in PE-immunized mice. *a*, Three-dimensional plot of the percentages of BrdU-labelled $B220^+PE^+$ spleen cells depending on the onset and the duration of BrdU-feeding. Each column represents a single experimental point, that is, the fraction of BrdU-labelled PE-binding cells isolated from pooled spleen cells of three mice. The position of the individual columns marks the beginning of BrdU-feeding after priming (from left to right) and the duration of BrdU-feeding (from front to back). The height of the column represents the fraction of BrdU-labelled cells among all PE-binding cells. The numbers depicted in the columns give the actual percentage of BrdU-labelled PE-binding cells. Spleen cells were analysed at the end of the labelling period as described in Fig. 1. *b*, Frequency of PE-binding splenic B-cells at different time points after priming. Background level of PE-binding cells in the spleen (unimmunized mice or mice primed with an unrelated antigen) is 5×10^{-6}. Each point represents the fraction of PE-binding cells in pooled spleen cells from three mice.

TABLE 1 *In vitro* secondary antibody responses of PE-binding and PE-nonbinding splenic B cells from PE-immunized mice

B cells	T cells	Anti-PE IgG1 antibodies (units)					
		Expt.	1	2a	2b	3a	3b
B220$^+$PE$^+$	—		*—	—	—	—	—
B220$^+$PE$^+$	CD4$^+$ cells		19	10	19	12	18
B220$^+$PE$^-$	—		—	—	—	—	—
B220$^+$PE$^-$	CD4$^+$ cells		—	—	—	—	—
—	CD4$^+$ cells		—	—	—	—	—

Spleen cells from PE-immunized mice were isolated 7 (experiment (expt) 2) or 12 weeks (expt 1 and 3) after priming, stained and enriched for IgM$^-$IgD$^-$ B cells as described in Fig. 1. B220$^+$PE$^+$ (expt 1, 250 cells per well; expts 2 and 3,500 cells per well) and B220$^+$PE$^-$ cells (expt 1, 7×10^3 cells per well; expt 2, 4×10^4 cells per well; expt 3, 8×10^4 cells per well) were sorted into 96-well microtitre plates as described elsewhere[7]. Cells were cultured in Mishell–Dutton medium with 10% FCS either in the presence or the absence of CD4$^+$ T cells of PE-immunized mice (2×10^5 cells per well). Splenic CD4$^+$ T cells were obtained by positive enrichment on a MACS[10] using the antibody GK1.5 (ref. 18) and were more than 90% pure. Supernatant collected 14 days after initiation of the culture was assayed for PE-binding IgG1 antibody by an enzyme linked immunosorbent assay using a biotinylated goat-anti-mouse IgG1 antiserum (Southern Biotechnology Associates) and 96-well microtitre plates coated with PE at a concentration of 5 μg ml^{-1}. In expts 2 ane 3, duplicate wells were analysed (a and b). The concentration of anti-PE antibodies is expressed in units ml^{-1}, 1,000 units ml^{-1} corresponding to the concentration of anti-PE antibody in a day-14 primary antiserum.

* <0·1 unit.

the persistence of B-cell memory requires the persistence of antigen, which is often stored on the surface of follicular dendritic cells for prolonged periods of time[15]. Such a dependence has been deduced from cell transfer experiments in which memory is rapidly lost if no antigen is added[4,16,17]. Possible cellular competition in occupying space in the irradiated recipient makes it difficult to compare this situation with that in the intact animal. The well-known superiority of live over killed vaccines might also indicate a need of recurrent antigenic stimulation for the maintenance of memory cells. However, this effect could also result from other mechanisms, such as prolonged recruitment of memory cells into either the T- or B-cell compartment. Taking all these points together, a role for persisting antigen in maintaining the long-lived memory B-cells which have been demonstrated here remains to be established. ☐

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β_2-Microglobulin restriction of antigen presentation

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ANTIGENS are generally thought to be recognized by cytotoxic T lymphocytes as peptides in the context of class I major histocompatibility proteins complex[1,2], which are heterodimers of heavy chains noncovalently associated with β_2-microglobulin (β2m). The highly polymorphic nature of the heavy chains and their resulting ability to present different sets of peptides has presumably evolved to allow potent immune responses against most pathogens. By contrast, the polymorphism of β2m is limited; seven alleles are known in the mouse[3,4] and only one has been identified in humans. β_2-Microglobulin was consequently thought to have only structural functions: namely, to ensure correct folding of class I molecules and their transport to the cell surface[5-9]. Although β2m is not implicated directly in the formation of the peptide binding site[10,11], we report here that it participates in the selection of MHC class I molecule-associated peptides.

DBA/2 (H-2^d, β2m^a) mice were immunized with P815 (H-2^d, β2m^a) mouse mastocytoma cells expressing the transfected mouse β2m^b gene. Cloned cytotoxic T lymphocytes (LAZ CTLs) showing specific lytic activity towards P815 cells transfected with mouse β2m were isolated. Experiments were performed to define the nature of the antigenic determinant recognized by these CTLs. They did not lyse P815 cells transfected with either the human β2m or a human × mouse hybrid β2m gene (Fig. 1), excluding, therefore, recognition of putative vector-derived T cell epitopes. Inhibition experiments with monoclonal antibodies specific for H-2K^d, D^d or L^d indicated that the eight isolated LAZ clones were H-2K^d restricted (Fig. 2). Surprisingly, these CTLs failed to lyse H-2$^{b/d}$, β2m$^{b/a}$ (C57BL/6 × DBA/2 F1) mouse lymphoblasts (Fig. 3*a*), as well as H-2^d, β2m^b (D2.1) B10.D2 mouse fibroblasts (Fig. 3*b*). These target cells were, however, efficiently lysed by control H-2K^d-restricted, HLA-Cw3-specific CAS 20 CTL clone (C.-A. Siegrist, unpublished observations).

These results led to the following hypotheses. First, the absence of lysis of C57BL/6 × DBA/2 F1 and D2.1 target cells (both expressing H-2K^d heavy chains and β2m^b) excluded the possibility that the LAZ CTLs recognized an antigenic determinant made up of the simple association of the H-2K^d heavy chains with the mouse β2m^b (as suggested previously by others, in the case of H-2K^b-restricted, β2m^b-specific CTLs, see refs 12, 13). This conclusion was reinforced by our consistent failure to sensitize H-2^d β2m^a target cells to lysis by incubation with purified mouse β2m^b under appropriate conditions for β2m exchange (data not shown). Second, these results argued against the recognition of a β2m^b-derived peptide in an H-2K^d-restricted manner. Such a peptide should have been produced and presented by the D2.1 target cells. In some cases, however, different types of cells process internal molecules in different ways[14]. This argument may explain why D2.1 cells escape LAZ CTL-mediated cytolysis (Fig. 2*b*), but does not apply to P815 cells transfected with the human × mouse hybrid β2m gene (Fig. 1*b*). Considering that β2m^a and β2m^b differ only at position 85 (an alanine being replaced by an aspartic acid)[15], that the usual size of the presented peptides is 10–20 amino acids and that the 30 C-terminal amino acids of the hybrid molecule are of mouse origin (Fig. 1*a*), one would have expected, as usually

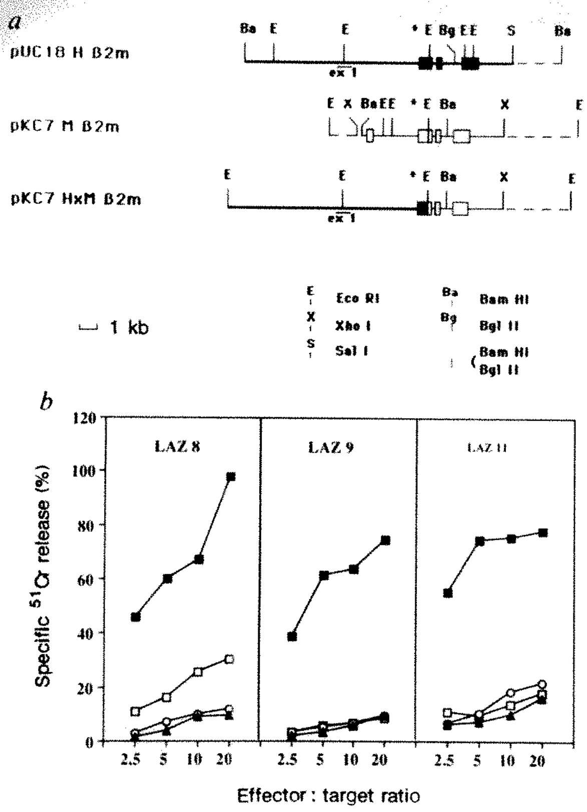

FIG. 1 Isolation of LAZ CTL clones and their reactivity on transfected P815 cells. *a*, Schematic representation of the transfected β2m genes. Plasmids pUC18 human β2m (pUC18 H β2m: exons represented as closed boxes)[24] and pKC7 mouse $\beta 2m^b$ (pKC7 M β2m, exons represented as open boxes)[25] were used for the construction of the hybrid β2m gene, taking advantage of an interspecies conserved *Eco*RI restriction site corresponding to amino acid position 70 (*E). The human × mouse hybrid β2m gene (pKC7 H×M β2m) was constructed by ligation between the *Eco*RI and *Xho*I sites of the pKC7 vector of the following 3 fragments: two *Eco*RI fragments of 7 and 4.3 kilobases (kb), isolated from the pUC18 H β2m plasmid, encompassing the promoter, leader sequence and 5' part of exon 2 of the human β2m gene and an *Eco*RI-*Xho*I fragment of 4.3 kb isolated from the pKC7 M β2m plasmid, containing the 3' end of the mouse $\beta 2m^b$ gene. Because of the multiplicity of most restriction sites this construct required several intermediate constructions which are not represented. The approximate location of the human β2m first exon (ex1) is indicated. P815 HTR (H-2^d, $\beta 2m^a$, high transfection rate) mastocytoma cells were transfected with each β2m plasmid together with the pMCS plasmid (containing the neomycin-resistance gene). Transfected cells were selected in the presence of geneticin (GIBCO) and cloned by limiting dilution. Human β2m-specific BE104 (γ2b, κ), mouse $\beta 2m^b$-specific S19.8 (γ2b) and IA^k-specific control 10-3.6 (γ2a, κ) mouse monoclonal antibodies were used at saturating concentrations for indirect immunofluorescence and FACS analysis of the selected transfected clones. The means of fluorescence with the three tested monoclonals were respectively 1.6, 156, 1.5 (P815 M $\beta 2m^b$), 93, 1.7, 3.2 (P815 H β2m) and 65, 1.9, 2.6 (P815 H×M β2m). *b*, Isolation of LAZ CTL clones. DBA/2 (H-2^d, $\beta 2m^a$) mice were injected intraperitoneally three times with 5×10^6 P815 transfected cells expressing mouse $\beta 2m^b$ (P815 M β2m). Spleen cells were first stimulated *in vitro* by transfected cells (30×10^6 responder cells, 1×10^6, P815 transfected cells (10,000 rad irradiated)) for 5 days and then restimulated (1×10^6 lymphoblasts from primary cultures, 10×10^6 DBA/2 feeder cells (2,000 rad irradiated) 1×10^6, transfected P815 cells (10,000 rad irradiated)) for 3 days. Surviving cells were subsequently cloned by seeding 4, 2 or 1 cells in round-bottomed 96-well microplates together with 10^5 feeder cells (2,000 rad irradiated) and 10^4 transfected P815 cells (10,000 rad irradiated). DMEM medium was supplemented with 10% FCS, 5×10^{-5} M 2-mercaptoethanol, 4.5 g l^{-1} glucose, 216 mg l^{-1} glutamine, 116 mg l^{-1} arginine, 36 mg l^{-1} asparagine, 10 mM HEPES buffer, 1 mM sodium pyruvate and 10% concanavalin A-stimulated rat spleen cell culture supernatant. Selected mouse $\beta 2m^b$-specific LAZ CTLs were tested in a 4 h ^{51}Cr release assay against P815 target cells untransfected (□), or transfected with human (▲), mouse (■), or human × mouse hybrid β2m (○).

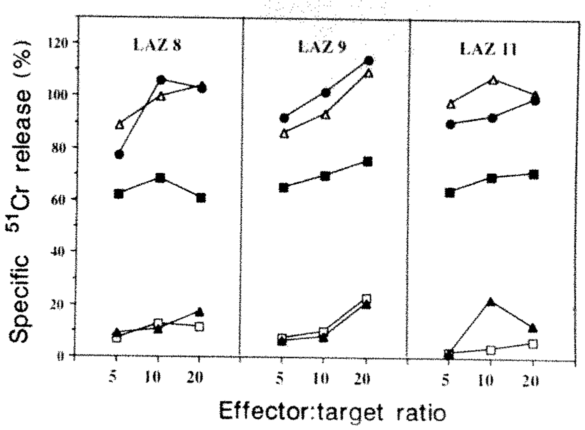

FIG. 2 H-2K^d restriction of LAZ CTLs lytic activity. Clones were tested in a 4 h ^{51}Cr release assay against untransfected P815 HTR cells (□) or P815 cells transfected with mouse $\beta 2m^b$ in the absence (■) or presence of 20-8-4S H-2K^d-specific (▲), 34-2-12 H-2D^d-specific (●) or 28-14-8S H-2L^d-specific (△) monoclonal antibodies, used as unpurified ascites diluted 1:50. Results are shown for three selected (LAZ 8, LAZ 9, LAZ 11) CTL clones.

observed with fusion proteins[16-18], that the $\beta 2m^b$-derived peptide (which should include position 85) would have been produced and presented by the P815 cells transfected with human × mouse hybrid β2m.

Therefore, we considered the possibility that LAZ CTLs recognize a P815 mastocytoma cell-specific peptide, whose presentation to the immune system is dependent on mouse $\beta 2m^b$. If this were the case, the peptide should be presented in exclusive association with $\beta 2m^b$ at the surface of $\beta 2m^b$-transfected P815 (H-2^d, $\beta 2m^a$) mastocytoma cells, a P815 cell extract treated with cyanogen bromide (CNBr), containing this peptide[19], should sensitize D2.1 ($\beta 2m^b$) target cells to lysis by H-2K^d-restricted LAZ CTLs and, also, one might expect some other peptides to exhibit differential affinity for the H-2K^d heavy chain depending on the associated mouse β2m allotype.

Experiments were performed to test these three predictions. The lytic activity of LAZ CTLs was inhibited by $\beta 2m^b$-specific monoclonal antibodies. HLA-Cw3-specific CAS 20 CTLs were not inhibited, as expected, as the pCw3 peptide is presented to the CAS 20 clone with equal efficiency in an H-2K^d × $\beta 2m^a$ or H-2K^d × $\beta 2m^b$ context (Fig. 4*a*). We also found that a CNBr-treated P815 cell lysate sensitized D2.1 ($\beta 2m^b$) but not P815 ($\beta 2m^a$) cells to lysis by LAZ CTLs. Both types of cell could, however, be sensitized to lysis by H-2K^d-restricted HLA-Cw3-specific CTLs using a similar extract from P815 cells transfected with HLA-Cw3 (Fig. 4*b*). Finally, a series of H-2K^d-binding peptides was tested as competitive inhibitors of the fixation of the pCw3 peptide to H-2K^d. One of them, pK^{d}174, was a more potent inhibitor of the fixation of the pCw3 peptide to H-2K^d × $\beta 2m^a$ (P815) than H-2K^d × $\beta 2m^b$ (D2.1) molecules (Fig. 4*c*). Taken together, these results strongly suggest that the LAZ CTL clones recognized a naturally produced P815-specific peptide, only bound and presented by $\beta 2m^b$-associated H-2K^d heavy chains.

The results reported here indicate that the structural polymorphism of mouse β2m influences (restricts) the presentation of peptides by MHC class I molecules to the immune system. Several contact residues have been identified between human β2m and the floor of the groove of the HLA-A2 and Aw68 class I heavy chains[10,11,20]; they do not include position 85. To explain the restricting effect of this residue on peptide binding, one might postulate either intramolecular interactions between distantly related residues or species-associated three-dimensional polymorphism of class I molecules. Alternatively, one might consider that class I molecules are flexible and can adopt configurations other than the one defined by crystallography, which might be critical for peptide binding.

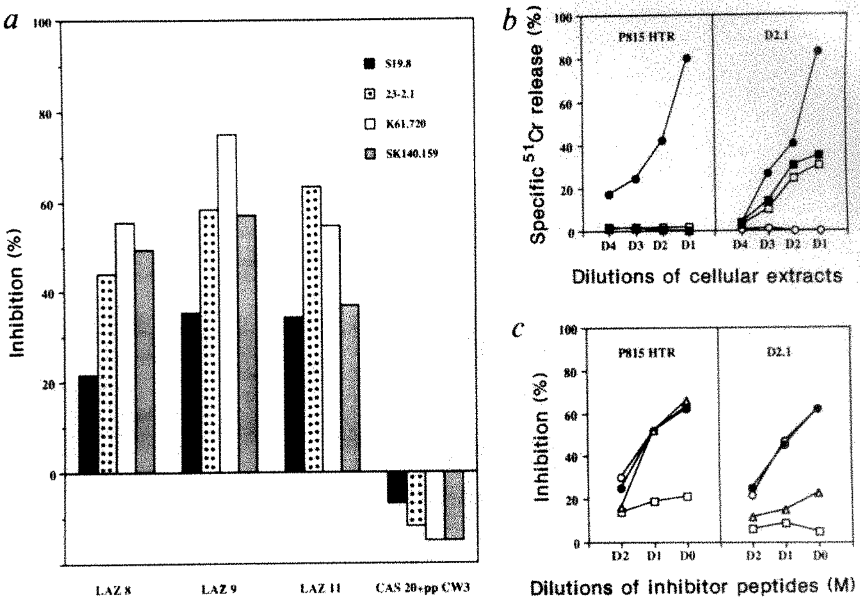

FIG. 4 β2m restriction of the fixation and presentation of peptides. *a*, Inhibition of LAZ CTLs by $\beta 2m^b$-specific monoclonal antibodies. H-$2K^d$-restricted $\beta 2m^b$-specific LAZ 8, LAZ 9 and LAZ 11 CTLs were tested against P815 M $\beta 2m^b$-transfected cells in the presence of four different $\beta 2m^b$-specific monoclonals used as unpurified ascites diluted 1:50. Similar experiments were performed using H-$2K^d$-restricted, HLA-Cw3-specific CAS 20 CTL, target cells being sensitized by addition of 1.8×10^{-8} M HLA-Cw3 (pCw3) derived 170–182 peptide. *b*, Sensitization of D2.1 cells to lysis by CNBr-treated cellular extracts. Extracts from 10^9 untransfected P815 (open symbols) or HLA-Cw3 × H β2m transfected P815 (closed symbols) cells were CNBr-treated[19]. Extracts were added to either P815 HTR ($\beta 2m^a$) or D2.1 ($\beta 2m^b$) H-2^d target cells at the onset of an otherwise standard ^{51}Cr release assay, starting from a 1.25 mg ml^{-1} final protein concentration and testing serial twofold dilutions. Susceptibility to lysis was evaluated using either H-$2K^d$-restricted $\beta 2m^b$-specific (squares) or H-$2K^d$-restricted HLA-Cw3-specific (circles) CTL clones. *c*, Peptide binding to H-$2K^d$ $\beta 2m^a$ or $\beta 2m^b$ target cells. Lysis (in the absence of inhibitor) of either P815 HTR (51%) or D2.1 (63%) target cells at 10:1 E:T ratio by H-$2K^d$-restricted, HLA-Cw3-specific CTLs in the presence of pCw3 peptide (RYLKNGKETLQRA 3.6×10^{-8} M final concentration (single-letter amino-acid code)), was evaluated in the presence of pB7 (RYLENGKDKLERA, □), pNP Arg⁻ (TYQRTRALVTG, ○), pQ10^b (RYELGKETLLRT, ●) or pK^d 174 (RYLENGNETLLRT, △) inhibitor peptides, starting from a 10^{-4} M final concentration and testing serial 10-fold dilutions. Results are expressed as percentage of inhibition.

a

	TARGET CELLS			
CTL CLONES	*B6 BLASTS H-2^b $\beta 2m^b$*	*DBA/2 BLASTS H-2^d $\beta 2m^a$*	*B6XDBA/2 BLASTS H-$2^{b/d}$ $\beta 2m^{b/a}$*	*P815 M β2m H-2^d $\beta 2m^{a/b}$*
LAZ 8	0	0	0	44.9
LAZ 9	0	0	0	47.8
LAZ 11	0	0	0	39.0
CAS 20-Cw3pp	0	0	0	1.6
CAS 20+Cw3pp	0	31.1	63.7	71.0

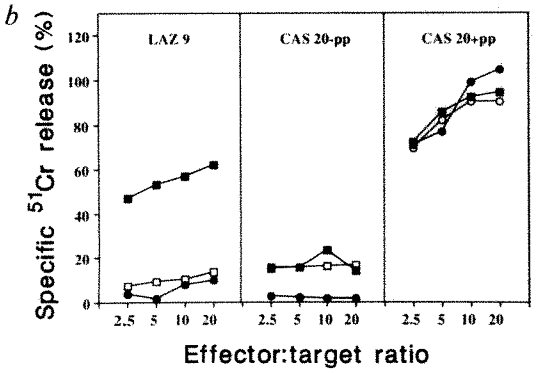

FIG. 3 Reactivity of the LAZ CTLs on different target cells. *a*, LAZ CTLs were tested at a 20:1 effector-to-target (E:T) ratio in a 4 h ^{51}Cr release assay against C57BL/6 (B6; H-2^b, $\beta 2m^b$), DBA/2 (H-2^d, $\beta 2m^a$), C57BL/6 × DBA/2 (B6 × DBA/2; H-$2^{b/d}$, $\beta 2m^{a/b}$) or target cells transfected with mouse $\beta 2m^b$ (P815 M β2m). Control H-$2K^d$-restricted HLA-Cw3-specific CAS 20 CTLs were tested at the same E:T ratio against the same target cells in the absence (−pp) or in the presence (+pp) of 1.8×10^{-6} M HLA-Cw3-derived peptide (amino acids 170–182). *b*, Two of the same CTLs were tested at 20:1, 10:1, 5:1 and 2.5:1 E:T ratio against untransfected (□) or mouse $\beta 2m^b$ transfected (■) P815 cells and H-2^d, $\beta 2m^b$ D2-1 (●) mouse B10.D2 fibroblasts.

To what extent β2m restricts antigen presentation is still unknown. β2m restriction of antigen presentation might explain control by the H-3 region (which includes the β2m gene amongst others) of the induction of H-$2D^b$-restricted H-Y antigen-specific CTLs[21]. Presentation of some peptides, however, seems to be independent of β2m allotype. For example, efficient presentation of the Cw3 peptide occurs whether H-$2K^d$ heavy chains are $\beta 2m^a$ or $\beta 2m^b$ associated (this study). Nevertheless, even a limited effect, combined with the extensive polymorphism of MHC class I heavy chains, should result in significant enlargement of the species repertoire of peptides presented by MHC class I molecules. At the same time, β2m polymorphism should result in the selection and presentation of different self peptides among individual mice, allowing the development of potent CTL responses and rapid allograft rejection. This could explain the strength of the H-3 minor histocompatibility locus. The reported results further indicate that the concept of minor histocompatibility antigen cannot only be restricted to the polymorphic endogenous molecules susceptible to processing into immunogenic peptides[22]. Other molecules[23] which may interact noncovalently with class I heavy chains might, like β2m, indirectly influence peptide binding. □

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Suppression of c-*ras* transformation by GTPase-activating protein

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THE *ras* genes are required for normal cell growth and mediate transformation by oncogenes encoding protein tyrosine kinases[1,2]. Normal *ras* can transform cells *in vitro* and *in vivo*, but mutationally activated *ras* does so much more efficiently, and highly transforming mutant versions of *ras* have been isolated from a variety of human and animal tumours (reviewed in refs 3–5). The *ras* genes encode membrane-associated, guanine nucleotide-binding proteins that are active when GTP is bound and inactive when GDP is bound[6–8]. The slow intrinsic GTPase activity of normal mammalian Ras proteins can be greatly accelerated by the GTPase-activating protein (GAP), which is predominantly cytoplasmic. This activity of GAP, which can increase with cell density in contact-inhibited cells[9], suggests that it functions as a negative, upstream regulator of *ras*. Other studies[10,11], however, show that GAP interacts with a region of *ras*-encoded protein implicated in *ras* effector function[12,13], which raises the possibility that GAP might also be a downstream target of *ras* (reviewed in ref. 14). Mutationally activated *ras*-encoded proteins also interact with GAP[15], although they are resistant to its catalytic activity[7]. In an attempt to define the role of GAP in *ras*-mediated transformation, we examined the effects on transformation of normal or mutant *ras* when cells overexpress GAP. We found that GAP suppresses transformation of NIH 3T3 cells by normal Ha-*ras* (c-*ras*) but does not inhibit transformation by activated Ha-*ras* (v-*ras*). These results support the hypothesis that GAP functions as a negative regulator of normal *ras* and make it unlikely that GAP alone is the *ras* target.

TABLE 1 Inhibition of c-Ras-induced foci by cotransfection with cloned GAP

	Experiment				
	1	2	3	4	Ave.
c-Ras/GAP	1	7	40	11	
c-Ras/control 1	28	—	96	89	
c-Ras/control 2	—	134	—	—	
% inhibition by GAP	96	95	58	88	84
v-Ras/GAP	530	268	208	151	
v-Ras/control 1	508	—	242	161	
v-Ras/control 2	—	394	—	—	
% inhibition by GAP	−4	32	16	6	13
v-Mos/GAP	314				
v-Mos/control 1	214				
% inhibition by GAP	−47				−47

GAP, pKZ61; control 1, pKZ93; control 2, pKZ75 (see Fig. 1). Transfections were carried out and foci scored as described previously[5]. In the various experiments, 25–100 ng per dish of Ras was used with 100–300 ng GAP plasmid or control plasmid. In experiments 1, 2 and 4, the Ras to GAP ratio was 1:3. In experiment 3, the ratio was 1:6. Ave., average.

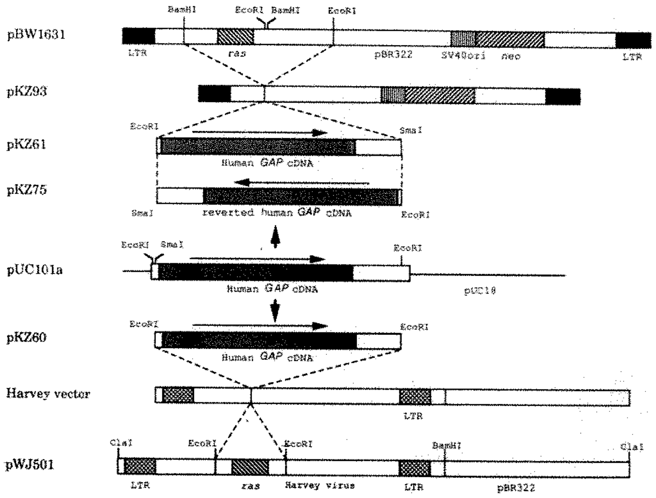

FIG. 1 Structure of plasmids with *GAP* and *ras*.

METHODS. Plasmid pBW1631, which contains c-*ras*, has been described previously[18]. The activated v-*ras* oncogene of Harvey murine sarcoma virus differs from c-*ras* only at codon 12 (encoding arginine instead of glycine) and codon 59 (encoding threonine instead of glutamine). Plasmid pKZ93, the vector without an insert, was constructed by digesting pBW1631 with *Bam*HI and *Eco*RI; the ends were made flush with Klenow fragment and religated. For pKZ61 and pKZ75, a full-length human *GAP* cDNA, from clone pUC101a, was digested with *Eco*RI and *Sma*I, the ends were made flush, and then ligated to the flush ended pKZ93 precursor. Plasmid pKZ61 contains the *GAP* cDNA in the sense orientation, pKZ75 in the antisense orientation. Plasmid pKZ60, which is a second *GAP* cDNA clone in the sense orientation, was constructed by substituting a *GAP Eco*RI fragment from pUC101a for the v-*ras Eco*RI fragment in pWJ1501. The construction of pWJ501 will be described elsewhere (W. M. Ju *et al.*, manuscript in preparation). It is Harvey murine sarcoma virus DNA cloned in pBR322 that has been modified to contain only two *Eco*RI sites, just upstream and downstream of v-*ras*, by substituting an *Eco*RI site for *Sac*II at nucleotide 940, substituting an *Eco*RI polylinker at the *Pst*I site at nucleotide 1,759, and removing the *Eco*RI site at nucleotide 2,540.

NIH 3T3 cells were transfected with normal or mutant *ras* genes placed under the control of a murine retroviral long terminal repeat. The human GAP complementary DNA clone[16], encoding the full-length 1,047-amino-acid GAP protein, was expressed from a Moloney murine leukaemia virus long terminal repeat in the mammalian expression vector pGV16 (refs 17, 18) (Fig. 1). Transfection of the NIH 3T3 line with normal c-*ras* resulted in the formation of a few dozen to over a hundred foci (Table 1). The presence of a three- to six-fold excess of pGV16 vector either bearing no inserted DNA (pKZ93) or with the *GAP* cDNA in the antisense orientation (pKZ75) did not appreciably inhibit the number of foci obtained with c-*ras* (Table 1; data not shown). However, when the plasmid pKZ61, encoding GAP in the sense orientation, was cotransfected with c-*ras* in a three- to six-fold excess, a striking inhibition in the number of foci was observed. The average inhibition in four independent transfections was 84% (Table 1). In contrast, the number of foci produced by the activated v-*ras* gene, which consistently results in a higher number of foci than c-*ras*, was not significantly affected by cotransfection with pKZ61. There was no inhibition of focus formation by v-*mos*[19], whose transforming activity is *ras*-independent[2], when it was cotransfected with the GAP-encoding plasmid (Table 1).

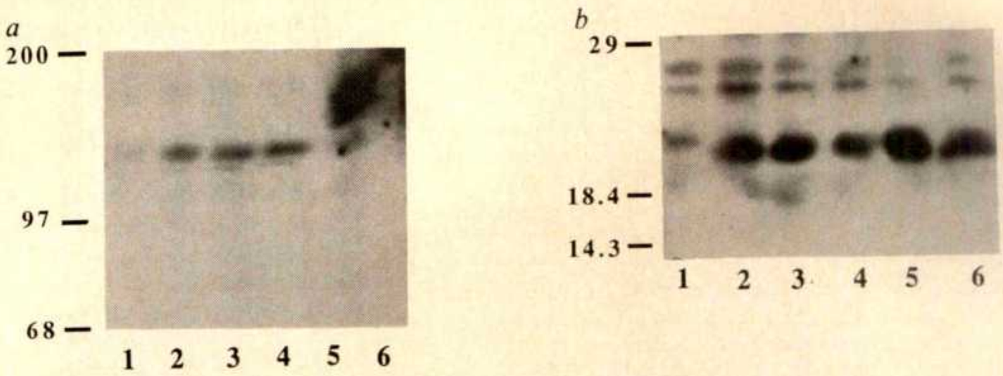

FIG. 2 Expression of GAP and Ras proteins in revertant and nonrevertant subclones. *a*, Level of expression of GAP protein as detected by western blotting with anti-GAP serum. Lane 1, normal NIH 3T3; lanes 2–4, revertant subclones 8-1, 8-2 and 8-4, respectively; lane 5, nonrevertant subclone 5-1T (isolated from c-*ras*-transformed cells transfected with the selectable marker alone); lane 6, nonrevertant subclone 8-10T (isolated from the same population as the revertant subclones). Arrow indicates GAP protein. *b*, Expression of Ha-c-*ras* p21 in cell lines as detected by western blotting with the anti-Ras monoclonal antibody 259. Lane 1, normal NIH 3T3; lanes 2–4, revertant subclones 8-1, 8-2 and 8-4, respectively; lane 5, nonrevertant subclone 5-1T; lane 6, nonrevertant subclone 8-10T. Arrow indicates Ras p21 protein.

METHODS. *a*, Confluent 100-mm plates were lysed in 28 mM Tris buffer, pH 7.1, 100 mM NaCl, 5 mM $MgCl_2$, 1% NP-40, 0.5% sodium deoxycholate, 1 mM DTT and 16 μg ml^{-1} aprotonin. Protein content of the lysates was determined using the bicinchoninic acid assay[31] (Pierce Chemical Co.), and 100 μg of each lysate was electrophoresed in an 8% SDS-polyacrylamide gel. Proteins were electroblotted onto an Immobilon membrane (Millipore Corp.), incubated with a polyclonal rabbit anti-GAP serum, then a goat anti-rabbit serum (Organon Teknika Corp.), and 10^7 c.p.m. ^{125}I-conjugated protein A (NEN Dupoint Co.). Autoradiography was for 7 days at −70 °C. Migration of molecular weight standards is shown on the left ($M_r \times 10^{-3}$). *b*, Western blotting was carried out as described for *a*, except that 20 μg protein was separated on a 15% polyacrylamide gel and the antibody incubations were with the rat monoclonal Y13-259 (ref. 32) followed by a rabbit anti-rat serum, then ^{125}I-conjugated protein A. Autoradiography was for 3 days at −70 °C. Migration of M_r standards is shown on the left ($M_r \times 10^{-3}$).

These data demonstrate that *GAP* can prevent transformation by c-*ras* but not by the activated v-*ras* allele. We did not see potentiation of c-*ras* transformation by *GAP*, as might have been expected if GAP alone were the *ras* target. The results raised the possibility that overexpression of GAP protein might be capable of inducing morphological reversion of cells already transformed by c-*ras*, but not by v-*ras*.

To test this idea, two NIH 3T3 lines transformed by c-*ras* and one v-*ras*-transformed line were cotransfected with an MuLV-based vector carrying the human *GAP* cDNA (Fig. 1, pKZ60) and a selectable marker, pSV2his or pSV2neo^r, conferring resistance to L-histidinol[20] or geneticin, respectively. Transfection of both c-*ras* lines yielded a large proportion of morphologically revertant resistant colonies when transfected with the *GAP* cDNA, and only a few when cotransfected with the control plasmid (Table 2). In contrast, there was no reversion of the v-*ras*-transformed line by the *GAP* cDNA.

TABLE 2 Morphological reversion of transformation by GAP

	Transformed cell lines % of flat colonies (no. revertants/total) c-*ras*	v-*ras*
Experiment 1		
Control	5 (3/56)	ND
GAP	20 (15/74)	ND
Experiment 2		
Control	1 (1/84)	1 (1/146)
GAP	85 (17/20)	2 (1/48)

Control, insertless Harvey vector; GAP, pKZ60 (see Fig. 1). One microgram of GAP or control DNA was cotransfected with 100 ng pSV2his plasmid in experiment 1. The same DNAs were cotransfected with 100 ng pSV2 neo in experiment 2. The c-*ras*-transformed clone used in experiment 1 was obtained by transfection with pBW1631, which contains *neo*^{r} as a linked marker, selection of geneticin-resistant colonies, and cloning of a transformant by end-point dilution. The c-*ras*-transformed clone used in experiment 2 was obtained by transfection of pCO7-FX (ref. 29), in which c-*ras* is under control of the Friend MuLV LTR, and selection of a transformant by end-point dilution. The v-*ras*-transformed clone was obtained by transfection with pKZ132 (in which the v-*ras* coding sequences have been inserted in the unique *Bgl*II site of pKSV-10 (ref. 30) by blunt-end ligation) and cloning by end-point dilution.

To confirm that the reversion of c-*ras* transformation was indeed due to expression of exogenous GAP, several drug-resistant subclones were isolated from among those analysed (Table 2, experiment 1). Northern blot analysis revealed that the revertant, but not the transformed, subclones expressed high levels of exogenous *GAP* RNA (data not shown). Cell extracts from the subclones were analysed by western blotting for GAP protein (Fig. 2*a*), which is predominantly cytoplasmic[7], although a proportion of GAP that has been phosphorylated on tyrosine in response to stimulation by platelet-derived growth factor is membrane-associated[21]. Under these assay conditions, the endogenous GAP protein of relative molecular mass 120,000 was barely detectable in NIH 3T3 cells (lane 1) or in c-*ras* transformants (lanes 5 and 6), whereas the revertants, as expected, contained much higher levels of GAP protein (lanes 2–4). These results confirm that the cells that were morphologically reverted by transfection of the *GAP* cDNA expressed significant levels of exogenous GAP protein. To exclude the possibility that the GAP-induced reversion might have resulted from a loss of c-*ras* expression, the cell lysates from the same set of subclones were examined by western blotting for the p21 protein encoded by *ras* with a Ras-specific monoclonal antibody. Parental NIH 3T3 cells expressed only a low level of p21, as expected (Fig. 2*b*, lane 1). In contrast, the five subclones derived from the c-*ras*-transformed line expressed abundant levels of p21 (lanes 2–6), whether they were morphologically transformed or revertant. Thus the level of GAP expression, rather than p21, correlates with the nontransformed state of the revertant subclones.

The findings presented here show that GAP can inhibit and reverse c-*ras* transformation of mammalian cells, whereas GAP had no detectable effect on transformation by the activated v-*ras* gene. The reversion of c-*ras* transformation by GAP indicates that an essential part of the *ras* transformation pathway in mammalian cells is overridden by the introduction of excess GAP into the cells. Thus the present results lend strong support for the role of GAP as an upstream negative regulator of *ras*, with GAP presumably functioning, through its catalytic activity, to reduce the level of active GTP-bound Ras protein. Consistent with this interpretation, a *GAP*-derived clone encoding only the C-terminal 340 amino acids, which constitutes the enzymatic domain of GAP protein[22], was also capable of suppressing c-*ras* transformation in these assay systems (unpublished observation). The results suggest that GAP as a negative regulator is limiting in NIH 3T3 cells and raise the possibility that overexpression of GAP might inhibit transformation by *ras*-dependent oncogenes, such as membrane associated tyrosine kinases[2].

If GAP alone represented the *ras* target, overexpression of GAP would be expected either to potentiate transformation or to have no influence on it. The inhibition of transformation by the presence of excess GAP is in stark contrast to the demonstration that overexpression of several proto-oncogenes, including c-*ras*, can induce transformation of established cells. The results indicate that GAP can act independently as a negative regulator of Ras activity, regardless of its possible downstream function.

The results obtained in mammalian cells are analogous to those recently reported for the biological activity of mammalian GAP in yeast[23]. In that study, mammalian GAP inhibited the function of c-*ras* but not mutationally activated *ras*. In addition, GAP functionally complemented yeast strains defective for the function of the *IRA*1 and *IRA*2 genes[23,24], which are upstream negative regulators of yeast RAS. But the study of GAP in yeast may not have allowed for an unbiased assessment of the role of GAP and its interaction with *ras*. GAP is not in question as the RAS target in yeast, and the known effector of RAS in yeast is adenylyl cyclase[25], which does not seem to be a target for Ras in higher eukaryotes[26,27].

Although our results tend to rule out the simple situation of GAP alone being the Ras target, they may not exclude the possibility that GAP might, in addition to being a negative regulator of Ras, participate in Ras target function if the target were a complex that involved molecules in addition to GAP. In this case, overexpression of GAP might inhibit *ras* transformation if a delicate stoichiometric balance required for the proper functioning of the hypothetical complex were disrupted by the presence of excess GAP in the cells. It may be relevant that GAP has recently been found to form a complex with two proteins that are not Ras in cells transformed by a variety of oncogenes that encode tyrosine protein kinases[28]. But the potential physiological significance of this association might be limited to GAP's function as an attenuator, and such complexes have not so far been identified in *ras*-transformed cells. Further analysis is required for a more complete understanding of the role of the Ras-GAP interaction in signal transduction. ☐

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Obligatory wounding requirement for tumorigenesis in v-*jun* transgenic mice

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AVIAN sarcoma virus 17 induces fibrosarcomas in chickens[1] and can transform a number of avian cell types *in vitro*[2] by the action of v-*jun* (refs 1–3). This gene and the related cellular genes *c-jun*[4–6], *jun* B[7] and *jun* D[8,9], encode transactivating (or repressing) DNA-binding proteins that form homo- or heterodimeric (Jun–Jun and Jun–Fos) complexes[5,10,11] which recognize the AP-1 consensus sequence TGACTCA (refs 12, 13), a response element that confers sensitivity to the tumour-promoting phorbol ester TPA[14]. We have produced several lines of transgenic mice carrying the v-*jun* oncogene, driven by the promoter of the widely expressed H–2K^k major histocompatibility complex (MHC) class I antigen gene. Transgenic animals are initially phenotypically normal, but after full-thickness wounding they show abnormal wound repair, characterized by hyperplastic granulation tissue. Many of these lesions are slowly progressive because of continuing fibroblast proliferation, and over 2–5 months some give rise to dermal fibrosarcomas. This reproducible multistep transition through a proliferative but benign intermediate is associated with characteristic increments in v-*jun* expression. Moreover, hyperplastic wound repair and its progression are both related to transgene dosage, suggesting that there exists a quantitative requirement or threshold for v-*jun* action. Our results indicate that v-*jun* is not oncogenic in transgenic mice as a result of a 'single-hit' mechanism, but rather, in addition to an obligatory wound, that secondary genetic or epigenetic events (possibly conscripting normal constituents of wound repair) are necessary for tumour development and progression.

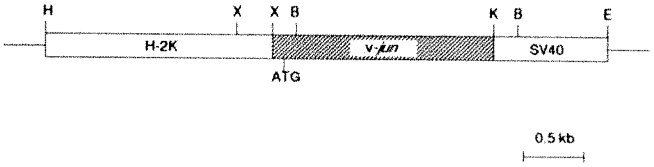


FIG. 1 Structure of the H–2K–v-*jun* transgene. The *gag–jun* fusion gene from ASV17 is driven by a 1.9-kb fragment from the 5′ end of the murine H-2K^k gene, ending 16 base pairs (bp) upstream of the H–2K translation initiation site. The polyadenylation signal is derived from the early region of SV40. Translation of v-*jun* mRNA begins at the initiation codon of the *gag* gene. H, *Hin*dIII; X, *Xba*I; B, *Bam*HI; K, *Kpn*I; E, *Eco*RI sites, respectively. ATG, *gag* initiation site.

METHODS. A 988-bp *Eco*RI–*Bcl*I fragment from SV40 was inserted between the *Eco*RI and *Bam*HI sites of a plasmid pSP64 derivative in which the *Xba*I site had been converted to a *Kpn*I site. The SV40 fragment was then retrieved as an *Eco*RI–*Kpn*I fragment, and inserted between the *Eco*RI and *Kpn*I sites of pG5-5-1, a pGEM4 derivative containing a 1.8 kb v-*jun* fragment from ASV 17 (ref. 1) cloned into the *Sma*I site, producing pv-*jun*PA. The 1.9-kb H–2K^k promoter segment[15] was isolated from pH2K^k as a *Hin*dIII–*Xba*I fragment and inserted between the *Hin*dIII and *Xba*I sites of the pv-*jun*PA intermediate to give pH-2K-v-*jun*. The final construct was digested with *Hin*dIII and *Eco*RI and a 4.7-kb fragment purified for microinjection into fertilized mouse eggs as described[33].

TABLE 1 Relative v-*jun* expression in H-2K-v-*jun* transgenic lines

Line	Th	Sp	H	Lu	Li	K	Br	Sk	Mu	Bo	Te	O	U	EF
Tg1	++++	+++	+	+++	+/−	+++	+/−	++	−	−	++	++++	++++	ND
Tg2	++++	+++	−	+/−	+/−	+/−	+/−	++	+/−	+/−	+++	−	−	++
Tg4	+++	+++	−	++	−	++	+	++	+/−	+/−	45+	ND	ND	ND

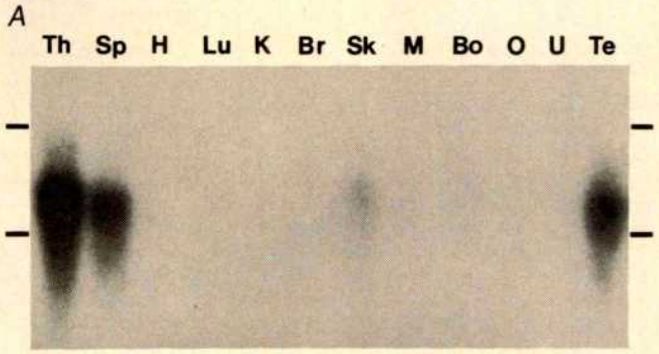
Steady-state levels of v-*jun* mRNA in a variety of transgenic tissues from hemizygous animals are shown. Tg1, Tg2 (C3H/C57Bl/6 hybrid background) and Tg4 (CD-1 background) contain respectively ~10, 150 and 50 copies of the transgene. The symbols +, ++, +++, ++++, represent linear increments in v-*jun* mRNA levels; +/−, trace; −, negative; ND, not done; Th, thymus; Sp, spleen; H, heart; Lu, lung; Li, liver; K, kidney; Br, brain; Sk, skin; Mu, skeletal muscle; Bo, bone; Te, testis; O, ovary; U, uterus; EF, homozygous primary embryo fibroblasts. In parallel experiments, v-*jun* expression was not detected in any non-transgenic tissues.

For northern analysis, 15 μg total RNA, prepared by the guanidinium isothiocyanate–caesium chloride method, was separated on a 1% agarose–formaldehyde gel, transferred to nitrocellulose and hybridized at 42 °C as previously described[32] to a random-primed v-*jun* probe (encompassing the entire 1.8-kilobase *gag–jun* fusion gene) in 25 mM KPO_4, pH 7.4, 5×SSC, 5×Denhardt's solution, 50% formamide, 50 μg ml^{-1} salmon sperm DNA and 10% dextran sulphate. Blots were first washed in 2×SSC, 0.1% SDS at 55 °C, and then sequentially in 0.2×SSC, 0.1% SDS at 55 °C and 65 °C. Quantitation of signal intensity in equally loaded lanes was by laser densitometry using a Molecular Dynamics 300A computing densitometer.

To investigate the oncogenicity of v-*jun* in multiple cellular lineages, we have generated several lines of transgenic mice carrying v-*jun* driven by the promoter of the widely expressed H-2K^k MHC class I antigen gene[15] (Fig. 1). Two lines of these H-2K/v-*jun* transgenic mice (Tg1 and Tg2) and an additional sterile founder (Tg4) have been studied in detail (Table 1). Constitutively expressed v-*jun* messenger RNA is detectable in varying amounts in a wide range of adult tissues, but with some differences in corresponding tissues among independent transgenic lines (Table 1; Fig. 2*a*). In general, v-*jun* expression is not related to transgene copy number, and there is a three- to fourfold difference in v-*jun* mRNA levels between high-expressing tissues such as thymus, spleen and testis and low-expressing tissues such as heart, liver, brain, muscle and bone.

H-2K-v-*jun* animals show no abnormalities in gross phenotype and a wide range of tissues from Tg1, Tg2 and Tg4 have been microscopically normal as well. A notable exception was the testes of the sterile Tg4, which were much smaller than those of control animals and microscopically showed a block to spermatogenesis at roughly the pachytene primary spermatocyte stage (not shown). The levels of v-*jun* mRNA in the testes of Tg4 were at least 15-fold higher than in those of fertile Tg1 and Tg2 mice (Table 1), suggesting that v-*jun* at comparatively high levels may block spermatogenesis. After a mean observation time of 8.5 months (up to 17 months in some cases), animals carrying H-2K-v-*jun* do not differ in spontaneous tumour incidence from non-transgenic strain-matched animals.

Although the transgenic animals are initially phenotypically



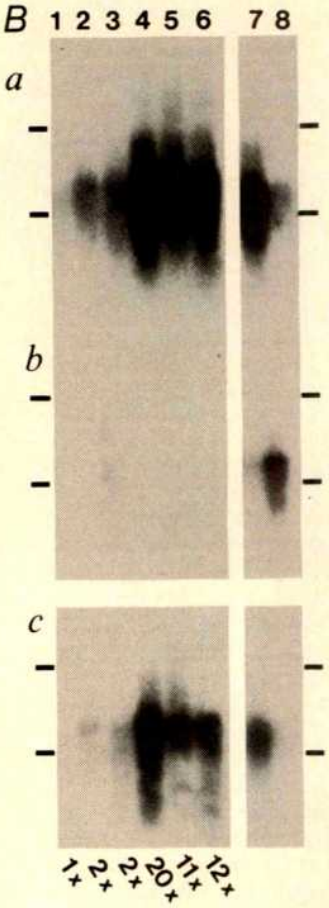
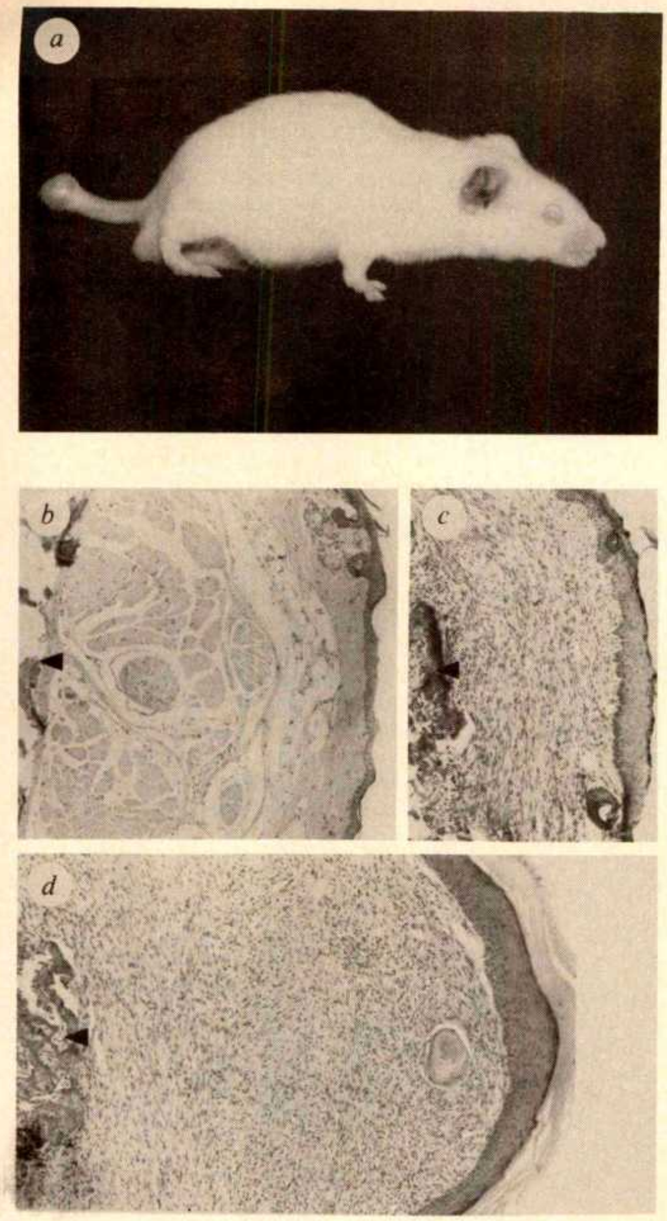


FIG. 2 Northern analyses of v-*jun* mRNA levels. *A*, Expression in representative hemizygous Tg2 tissues. The 2.2-kb v-*jun* transcript was detected with a v-*jun* probe. The migration positions of 28S and 18S ribosomal RNAs are indicated. Th, thymus; Sp, spleen; H, heart; Lu, lung; K, kidney; Br, brain; Sk, skin; M, skeletal muscle; Bo, bone; O, ovary; U, uterus; Te, testis. Methods were as described in Table 1. *B*, Expression during progression from abnormal wound to fibrosarcoma. Lane 1, normal Tg2 tail shaft; lane 2, 8 pooled 10-day hyperplastic wounds from Tg2; lane 3, 8 pooled Tg2 intermediate lesions 2–3 months after wounding; lane 4, Tg4 tail fibrosarcoma 3 months after wounding; lanes 5 and 6, 2 Tg2 tail fibrosarcomas, both 3 months after wounding. All Tg2 samples are from homozygous animals. Lanes 7 and 8, Tg4 testis and urinary bladder, respectively, taken from the same filter as lanes 1–6. The positions of 28S and 18S rRNAs are marked. Relative amounts of v-*jun* mRNA are indicated below *c*. *a–c*, Sequential hybridization with v-*jun*, rat c-*fos* and avian retroviral *gag*-specific probes, respectively. The *gag* probe recognizes v-*jun* transcripts exclusively. In theory, the v-*jun* probe can hybridize with murine c-*jun* mRNAs (expected at 2.7 and 3.2 kb), but we were unable to detect these species in any of the samples analysed under the hybridization conditions used. Neither the *gag* nor v-*jun* probes detect expression in non-transgenic tissues. Results identical to *a* and *c* were also obtained with a conserved *jun* leucine zipper–DNA-binding domain probe (not shown). Bladder RNA from Tg4 served as a positive control for c-*fos* expression.

METHODS. RNA was prepared and analysed as in Table 1. *a*, The v-*jun* probe is identical to that used in Table 1. *b*, The c-*fos* probe is a 1.3-kb *Nae*I fragment from rat c-*fos* cDNA[34]. *c*, The *gag*-specific probe is a 0.8-kb *Sst*I–*Nco*I fragment from pIV2.3TO3, a Fujinami sarcoma virus derivative (gift from M. Moran). The leucine zipper–DNA binding domain probe is a 279-bp fragment derived from the C terminus of v-*jun*, encompassing nucleotides 1,269–1,548 (ref. 1).



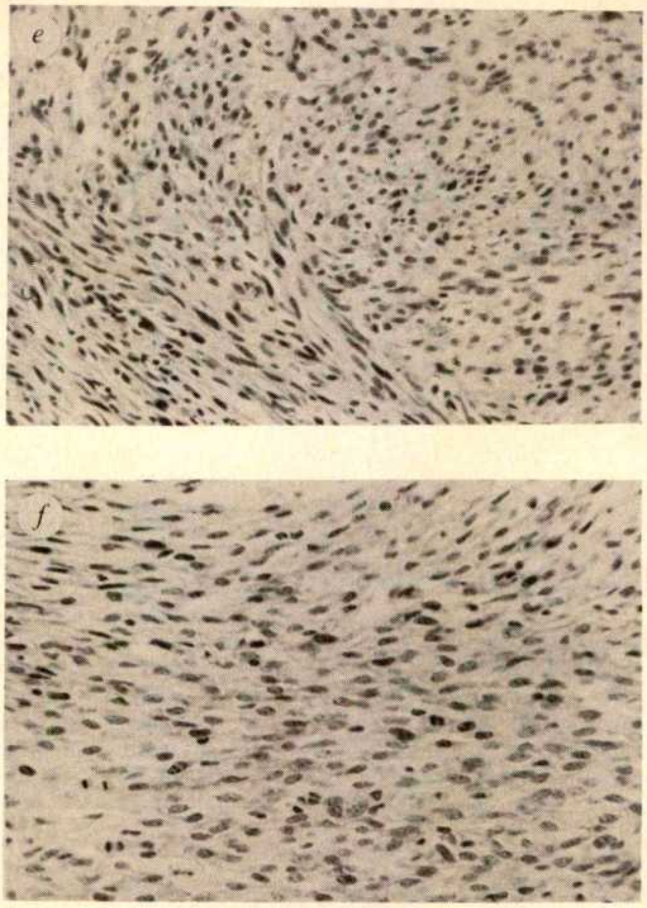
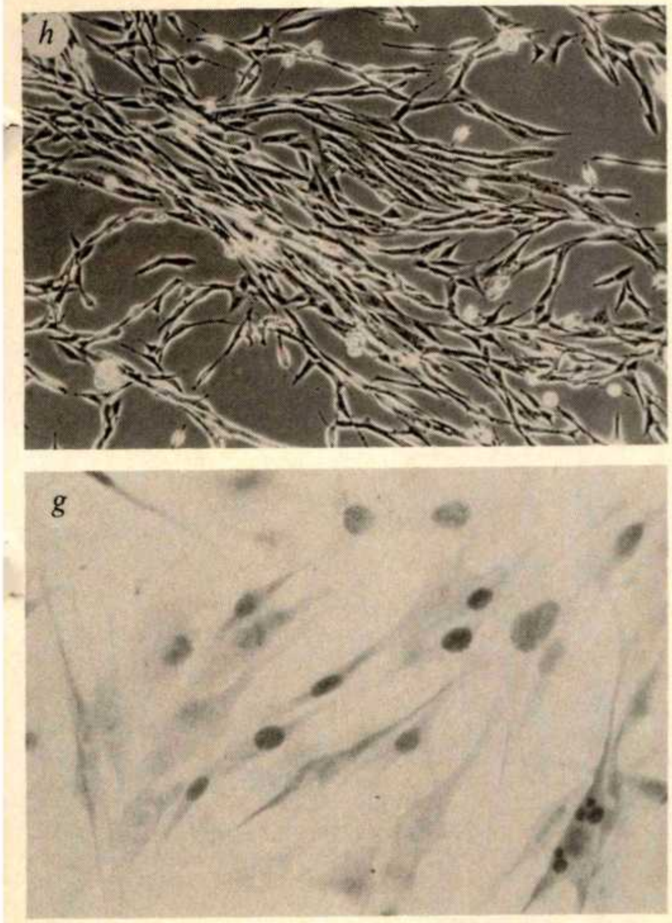


FIG. 3 Representative examples of H-2K-v-*jun* wound-related pathology and tumour-derived cell lines. *a*, H-2K-v-*jun* sarcomas of tail and ear in Tg4, 3 and 5 months after wounding, respectively. *b*, Cross-section of unwounded non-transgenic tail showing normal thickness of epidermal and dermal layers on far right and extensive underlying muscle and fat. Arrow at left marks position of bone (×53). *c*, Two-week tail wound from non-transgenic animal showing repairative dermal tissue composed primarily of granulation tissue fibroblasts and reactive hyperplasia of the overlying epidermis (×53). *d*, Two-week tail wound from homozygous Tg2 animal, illustrating marked increase in granulation tissue (×53). In *c* and *d*, arrows mark the position of underlying bone. *e*, Intermediate progressive lesion from homozygous Tg2 animal 10 weeks after wounding, showing extensive but benign fibroblastic proliferation (×170). *f*, Well-differentiated dermal fibrosarcoma from homozygous Tg2 animal, 15 weeks after tail wounding. Comparison with *e* reveals increased cellularity and mitotic index, increased nuclear pleomorphism, prominence of nuclear membranes and of nucleoli, and increased coarseness of chromatin (×170). *g*, Morphology of cell line from Tg4 tail fibrosarcoma (phase contrast, ×113). *h*, Nuclear localization of v-jun by immunocytochemistry in cells derived from a Tg2 tail fibrosarcoma. Heterogeneous staining is consistently observed in clonally-derived

normal, a proportion of animals exhibits abnormal wound repair consisting of circumferential (and occasionally more focal) wound hypertrophy, following full-thickness wounding such as that which results from tail-cutting or ear-punching. This abnormality is usually unmistakable by 10–14 days following injury, and consists microscopically of hyperplastic granulation tissue (Fig. 3*d*) in large excess of that found in an identically produced, but non-transgenic wound (Fig. 3*c*). In many cases, abnormal growth ceases when the tissue defect is completely repaired but in others, the lesions continue to grow slowly for months, during which histological analysis reveals a benign but progressive dermal fibroblastic proliferation (Fig. 3*e*). Ultimately, a proportion of these intermediate lesions increases in size much more rapidly and becomes centrally haemorrhagic (Fig. 3*a*), and microscopic analysis is consistent with a diagnosis of well-differentiated fibrosarcoma (Fig. 3*f*). These malignancies can be locally invasive, but have not been observed to metastasize. The location and extent of wounding play a critical part in eliciting this phenotype. Abnormal wound repair and its subsequent progression have been observed primarily following wounds that completely or partially transect the ear or tail, but never after superficial epithelial injuries, suggesting that dermal (and possibly subdermal) injury or larger tissue defects that can be repaired only by extensive fibroblast proliferation are particularly favoured for this outcome.

Tumorigenesis following wounding has been observed in all three transgenic lines, but with marked differences in frequency. In the Tg4 founder, the progression from abnormal tail wound to sarcoma occurred repeatedly after sequential tail cutting. In the Tg1 line, abnormal wound healing is observed in <5% of cases and so far, only one of these wounds has progressed to sarcoma. In Tg2, however, the entire sequence is observed frequently and predictably. Moreover, within this pedigree the wounding phenomenon and its subsequent progression are related to transgene dosage: After at least 3 months follow-up, in 50 hemizygous animals, 26 abnormal wounds (52%) gave rise to 10 slowly progressive lesions (38%), among which 4 sarcomas have been observed to occur at a mean of 20 weeks after



tumour cell populations.

METHODS. *b–f*, Tissue samples were fixed in 10% formaldehyde and then processed routinely for standard haematoxylin-eosin staining of 5-μm paraffin sections. *g*, Sarcoma samples were minced finely and plated in RPMI 1640 or DME-high glucose medium supplemented with 10% FCS, 2 mM glutamine, 100 U ml^{-1} penicillin, 100 μg ml^{-1} streptomycin, and 0.25 μg ml^{-1} amphotericin B. Medium was replaced twice weekly, and after 3 weeks, adherent cells extending from the tumour fragments were disaggregated with 0.05% trypsin/0.53 mM EDTA and replated. Clonal lines were subsequently established by limiting dilution. *h*, Cells were seeded on glass coverslips and incubated overnight in DME-high glucose medium supplemented as above, fixed in 4% paraformaldehyde in 10 mM PBS (10 min) and permeabilized in −20 °C acetone for 3 min. They were then rinsed in 50 mM Tris (pH 8.0), 150 mM NaCl and stained immunocytochemically using an avidin-biotinylated horseradish peroxidase kit (Vectastain ABC) with 0.05% diaminobenzidine tetrahydrochloride as peroxidase substrate and monoclonal anti-*gag* antibody, R254E (gift from T. Pawson) as primary antibody. This antibody detects the *gag* portion of v-Jun which is absent from cellular Jun proteins. Coverslips were then counterstained lightly with eosin.

wounding. By contrast, in 27 homozygous animals, 24 abnormal wounds (87%) gave rise to 18 intermediate lesions (75%), among which 6 sarcomas have been observed, occurring at a mean of 14 weeks following wounding. Thus, not only are abnormal wound repair and its progression more frequent in homozygous animals, but progression to sarcoma also occurs more rapidly.

Reproducible differences in transgene expression are observed in parallel with the morphological and corresponding histological progression from abnormal wound to sarcoma (Fig. 2*b*): v-*jun* mRNA is detectable at low levels in normal tail shaft, as well as in other tissues that may be found in tail such as skin, muscle, bone, and fibroblasts (Table 1). In 10–14 day-old hypertrophic wounds, there is roughly a twofold increase in v-*jun* mRNA, which persists in intermediate lesions 2–3 months old (which by this time may be ⩾0.5 cm in diameter). The subsequent conversion to sarcoma, however, is accompanied by an additional 5–10-fold increase in v-*jun* mRNA. The progression from abnormal wound to sarcoma, is not accompanied by any detectable changes in c-*fos* mRNA (Fig. 2*b*), nor could we demonstrate any accompanying changes in c-*jun* mRNA levels.

Several clonal cell lines have been established from Tg2 and Tg4 tail sarcomas (Fig. 3*g*). These cells are pleomorphic in appearance and include a component that can spontaneously give rise to multinucleated structures resembling myotubes (not shown). In addition, these cells form colonies in soft agar, are tumorigenic in nude mice (not shown), and can readily be shown to express v-*jun* by northern RNA analysis and by immunocytochemistry (Fig. 3*h*).

Our observations on tumour development and progession in H-2K-v-*jun* transgenic mice may provide some clues regarding the oncogenicity of v-*jun*. First, as only tail and ear fibrosarcomas have been observed, even though v-*jun* is expressed in a variety of tissues, all cell types may not be equally susceptible to transformation by v-*jun*, at least at the levels of expression attained. Second, v-*jun*-induced hyperplasia seems to depend on a transient wound-derived component. Wounds are known to mobilize a variety of growth factors and cytokines[16–21] that may be interacting with v-Jun protein or with the transgene itself[22–24] to induce proliferation. Third, the obligatory requirement for tissue injury and repair to initiate tumorigenesis indicates that even in tissues susceptible to v-*jun*, v-*jun* is not oncogenic as a 'single hit.' Indeed, in addition to the initiating wound, tumorigenesis probably requires early and late secondary genetic or epigenetic events that initially allow autonomous rather than wound-regulated proliferation, and later lead to increased v-*jun* expression. Fourth, the striking difference in wounding response between Tg1 and Tg2, despite similar v-*jun* mRNA levels in skin, muscle and bone, suggests that some other pedigree-specific feature of v-*jun* expression, such as inducibility within a wound or a subtle difference in cellular range of transgene expression, may be critical for tumorigenesis. Finally, and most intriguing, are the high levels of v-*jun* expression in sarcomas which imply distinct quantitative requirements or thresholds for v-*jun* action in early and late lesions.

The obligate relationship between wounding and appearance of tumours in H-2K-v-*jun* transgenic mice is consistent with anecdotal reports of this phenomenon in humans[25–26] and with previous studies in birds[27] and mammals[28]. Wound-associated mesenchymal neoplasms are also seen in transgenic mice carrying the bovine papilloma virus type-1 genome[29–31]. The long latencies of tumour formation in these mice, however, suggest that these neoplasms, unlike those of H-2K-*v-jun* mice, have not arisen directly as an immediate outgrowth of the wound itself. The reproducibility of the wounding phenomenon in the H-2K-*v-jun* mice provides a useful model for studying tumour development and progression. Not only can the multistep conversion of an abnormality of a benign physiological process to frank malignancy be easily and accessibly studied, but the ability to isolate and analyse tissue from discrete stages in this sequence may allow identification of changes that lead to tumour progression, and how they may relate to normal components of tissue repair. Haddow proposed that "The wound may be regarded as a tumour which heals itself"[25]: the v-*jun*-induced neoplasms that we describe reinforce this view by illustrating its corollary—the tumour as a wound that never heals. □

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Human cyclin A is adenovirus E1A-associated protein p60 and behaves differently from cyclin B

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CYCLINS are proteins synthesized during each cell cycle and abruptly destroyed in each mitosis[1-5]. Cyclins have been implicated in the induction of mitosis[6,7] and are associated with the serine–threonine protein kinase p34^{cdc2} as components of mitosis promoting factor (MPF)[8-10]. On the basis of conserved sequence motifs cyclins can be divided into A or B types. We recently cloned a human cyclin B (ref. 11) and showed that cyclin B expression is regulated transcriptionally and post-translationally during the cell cycle, and that cyclin B associates with p34^{cdc2}. Here we report that human cyclin A messenger RNA and protein levels also vary during the cell cycle, and increase and decrease in advance of cyclin B levels. Cyclin A is associated with a protein of relative molecular mass 33,000 that is related to, but distinct from, p34^{cdc2}, and this complex has histone H1 kinase activity *in vitro*. Cyclin A is identical to p60, a protein that associates with p34^{cdc2} in interphase cells[12] and with adenovirus E1A in transformed cells[22].

We isolated a human cyclin A complementary DNA using the polymerase chain reaction (PCR). Degenerate oligonucleotides to two conserved cyclin A motifs were designed on the basis of the known cyclin A sequences from *Drosophila*[13], *Spisula*[2] and *Xenopus* (T. Hunt, personal communication). As template we used the HeLa cell cDNA library[14] from which we had isolated a cyclin B cDNA clone. Using the cloned PCR product as a probe, we isolated a 1.4-kilobase (kb) cDNA and in turn used this clone to isolate a 2.2-kb cDNA from a human keratinocyte cDNA library. Recently a human cyclin A cDNA has been independently cloned[15] and the amino-acid sequence reported is identical to our own. We detected a single human cyclin A mRNA of about 2.2 kb; it is not clear why this is apparently smaller than the main 2.7-kb cyclin A cDNA previously reported[15].

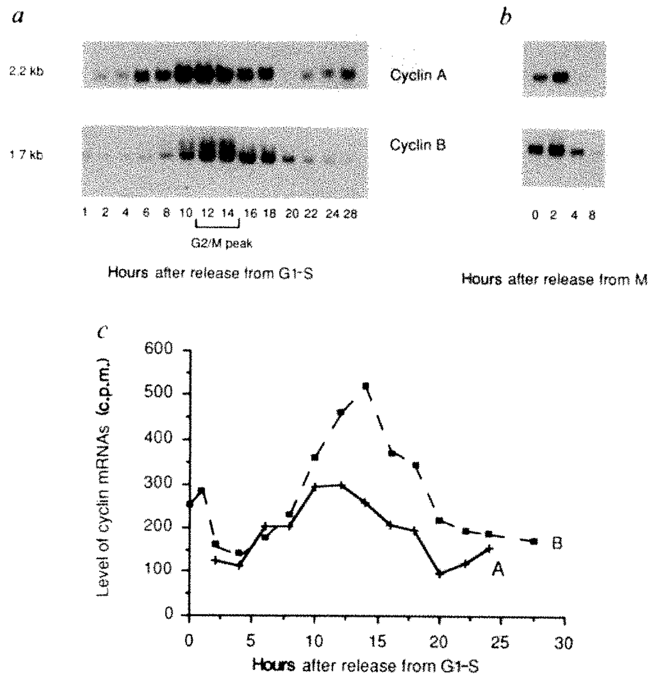

FIG. 1 Cyclin A and B mRNA levels vary during the cell cycle. *a*, Northern blot of RNA from cells after release from a G1–S phase block, probed with cyclin A and B cDNAs. Cells were synchronized by a thymidine–aphidicolin block and RNA was prepared at the indicated times as described[11]. The blots were probed with M13 sequencing clones of either cyclin A or B prepared by primed-cut labelling. Flow cytometry showed that the M-phase peak was at 12–14 h. RNA sizes are given in kb on the left. *b*, Northern blot of RNA from cells after release from a mitotic block. RNA was prepared and blotted as above, at the indicated times, from cells released from a nocodazole block[11]. The apparent increase in cyclin mRNAs after the release from nocodazole block is a loading artefact. When the cyclin mRNA levels are normalized to actin mRNA they remain constant for 2 h after removal of nocodazole and then begin to decline. *c*, Relative intensity of cyclin A and B mRNA signals from the blots shown in *a* as measured by a beta scanner and normalized to that of actin[11].

METHODS. RNA was prepared from ~8 × 10^6 HeLa cells per time point. The same RNA samples were used for the cyclin A and B blots in *a* and *b*. Total RNA was loaded (10 μg per lane) on a 1% agarose–formaldehyde gel and the RNA transferred and probed as described[11]. The cyclin A blot was exposed with an intensifying screen for 3 h at −70°C, and the cyclin B blot for 16 h at room temperature without a screen. RNA markers (BRL) were run in parallel.

We find that the level of cyclin A mRNA is regulated in the cell cycle. In G2 there is roughly three times more cyclin A mRNA than in G1, and this reaches its maximal level 2 h before the peak in the amount of cyclin B mRNA (Fig. 1*a*). The rate of transcription of cyclin A mRNA also increases about 2 h before that of cyclin B as determined by nuclear run-off experiments (data not shown). In addition to an increased rate of transcription, cyclin A mRNA may be more stable in G2 than in G1. The level of cyclin A mRNA declines more rapidly after mitosis than that of cyclin B (Fig. 1*a*, *b*), implying that cyclin A mRNA is more unstable than cyclin B mRNA in G1, consistent with the presence of several AUUUA motifs in its 3′ untranslated region, which are not found in cyclin B mRNA[11].

The cyclin A *in vitro* translation product has an apparent relative molecular mass (M_r) of 58,000 (58K), as determined by SDS-PAGE (data not shown). This, coupled with the histone H1 kinase activity of cyclin A immunoprecipitates (see below), indicated that cyclin A could be related to the p60 protein that associates with p34^{cdc2} (ref. 12). In support of this we found that the cyclin A *in vitro* translation product was immunoprecipitated with the anti-p60 monoclonal antibody C160 (ref. 12). Furthermore, the [^{35}S]methionine-labelled tryptic-peptide map of the *in vitro* translation product of cyclin A is identical to that

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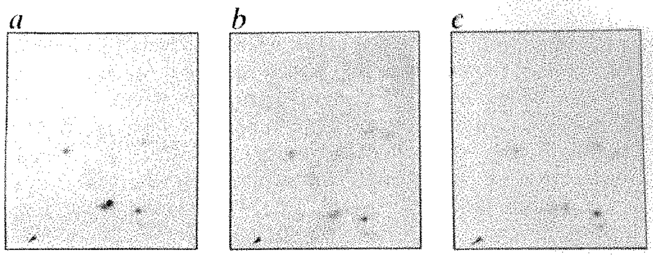
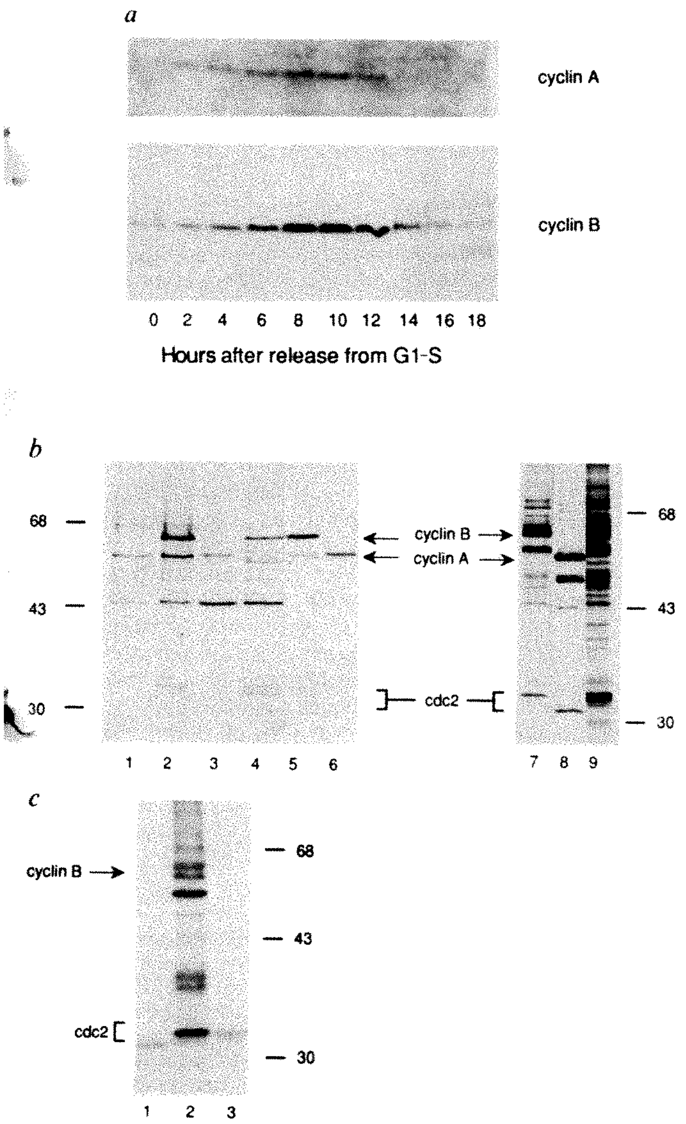
FIG. 2 Cyclin A is identical to p60. *a*, [^{35}S]methionine tryptic peptide map of cyclin A *in vitro* translation product (8,500 c.p.m. applied, exposed for 72 h). *b*, [^{35}S]methionine tryptic peptide map of p60 immunoprecipitated from HeLa cells with monoclonal antibody C160 (950 c.p.m. applied, exposed for 500 h). *c*, [^{35}S]methionine tryptic peptide map of mix of samples from *a* and *b* (800 c.p.m. of each sample applied, exposed for 500 h).

METHODS. Cyclin A synthetic mRNA was transcribed and translated *in vitro* from an SP6 promoter as previously described[11]. Roughly 2×10^6 HeLa cells were labelled for 2 h in methionine-free DMEM + 10% dialysed fetal bovine serum supplemented with 0.5 mCi ml^{-1} [^{35}S]methionine (Amersham). Cells were lysed in 1 ml RIPA buffer and 10 μl monclonal antibody C160 cell supernatant used to immunoprecipitate p60 per 100 μl lysate. Tryptic peptides were prepared and run as two-dimensional maps by electrophoresis at pH 1.9 in the horizontal direction (anode on the left), and by ascending chromatography[11]. The origin is marked by an arrow.





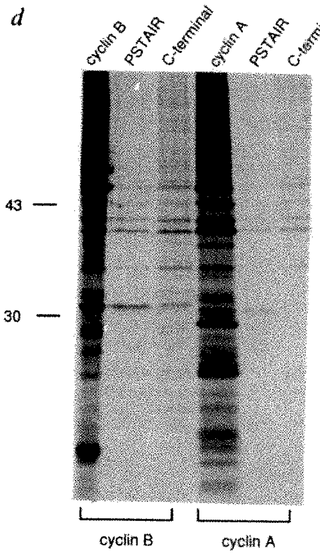


FIG. 3 Cyclin A and B protein levels alter during the cell cycle and co-immunoprecipitate with different ~34K proteins. *a*, Immunoblot. HeLa cells were released from an aphidicolin block and total cell lysates were prepared in SDS lysis buffer at the indicated times. Equal amounts of protein were loaded per lane. The blot was probed with either anti-cyclin A or anti-cyclin B antiserum followed by ^{125}I-labelled protein A as previously described[11]. Immunoblots were exposed at −70 °C with an intensifying screen for 150 h (cyclin A) or 15 h (cyclin B). Flow cytometry showed the G2–M peak to be at 14 h. *b*, Immunoprecipitations from [^{35}S]methionine/cysteine-labelled HeLa cells. Lane 1: cyclin B, preimmune serum; lane 2: anti-cyclin B (exposed for 12 h); lane 3: anti-cyclin A (exposed for 14 h); lane 4: anti-cdc2 C-terminal peptide (exposed for 14 h); lane 5: p13^{suc1}-Sepharose eluate (exposed for 14 h); lane 6: anti-cyclin A (exposed for 14 h); lane 7: anti-cyclin B (exposed 12 h); lane 8: anti-cyclin A (exposed 5 days); lane 9: longer exposure (5 days) of lane 7. M_r markers (in K) are on the left and right. Lanes 1–6, and lanes 7–9 were run on separate gels. Lanes 4 and 9 show the positions of all three forms of p34^{cdc2}. *c*, Immunoprecipitation of cyclin A (lane 1), cyclin B (lane 2) and p34^{cdc2} (lane 3) from ^{32}P-labelled HeLa cells. The gel was exposed for 15 h with an intensifying screen at room temperature. M_r markers (in K) are on the right of the figure. *d*, Re-immunoprecipitation of the 33–34K proteins from ^{35}S-labelled anti-cyclin A or anti-cyclin B immunoprecipitates with either an anti-PSTAIR or an anti-p34^{cdc2} C-terminal peptide antibody. The exposure shown was for 40 h, but much longer exposures confirm that the cyclin A-associated 33K protein is not re-immunoprecipitated with the C-terminal antibody.

METHODS. For immunoblots ~0.5×10^6 cells were lysed in 100 μl SDS lysis buffer per time point. The concentration of protein in each sample was measured by the BCA protein assay (Pierce Chemicals) and 33 μg of each sample was loaded per lane. Polyclonal anti-cyclin A antisera were raised in rabbits to a bacterially expressed fusion protein of the TrpE protein fused to the C-terminal 46 amino acids of cyclin A. For immunoblots, anti-cyclin A antiserum was used at a final concentration of 1:250, anti-cyclin B antiserum was used at 1:1000. An extra wash in 0.1% Nonidet P-40, 150 mM NaCl, 10 mM Tris-HCl buffer, pH 7.4, removed the signal from the ~58K protein previously recognized by anti-cyclin B antibodies[11]. The signals obtained for cyclin A and B on immunoblots are not equivalent because the titres of the antisera differ. Asynchronously growing cells were labelled for 2 h with [^{35}S]methionine/cysteine or for 3 h with ^{32}P-orthophosphate under conditions previously described[11]. Cells were lysed in RIPA buffer and cyclin B immunoprecipitations were prepared and analysed as previously described[11]. For cyclin A immunoprecipitations, 10 μl monoclonal antibody C160 cell supernatant was used per 100 μg cell lysate. For p34^{cdc2} immunoprecipitates, 1 μl anti-p34^{cdc2} C-terminal peptide antiserum was used per 100 μg of cell lysate. The same ^{35}S-labelled lysates were used for all the immunoprecipitations in *b*, and the samples were run on the same gel. Re-immunoprecipitations of the 33–34K proteins from cyclin A or B immunocomplexes were carried out as described[4].

of p60 immunoprecipitated with C160 (Fig. 2). We conclude that p60 is cyclin A.

Antibodies raised to a bacterially expressed cyclin A fusion protein immunoprecipitated a 60K protein that had an identical peptide map to p60 (data not shown). We used these antibodies and the C160 monoclonal antibody to study the level and activity of cyclin A through the cell cycle compared with cyclin B. By immunoblotting we found the amount of both cyclin A and B increases through the S and G2 phases to a maximum at mitosis, at which point they are degraded (Fig. 3*a*). Cyclin A destruction seems to be initiated before that of cyclin B (Fig. 3*a*), which is in keeping with the behaviour of the cyclins in clams, where cyclin A is degraded first in metaphase, and cyclin B later at the metaphase–anaphase transition[4,16,17].

Both cyclin A and cyclin B co-immunoprecipitate with proteins of around 34K from either ^{35}S- or ^{32}P-labelled cells (Fig. 3*b* and *c*). We have previously identified by tryptic peptide mapping the 34K protein associated with cyclin B as $p34^{cdc2}$ (ref. 11). As expected, this 34K protein is recognized by both an anti-C-terminal $p34^{cdc2}$ peptide antibody and by an antibody raised against the conserved PSTAIR motif (one-letter amino-acid notation) when released from an anti-cyclin B ^{32}P-labelled immunoprecipitate (Fig. 3*d*). By contrast, cyclin A seems to be mostly associated with a ~33K protein related to, but distinct from $p34^{cdc2}$. The ~33K protein associated with cyclin A migrates slightly ahead of all three forms of $p34^{cdc2}$ immunoprecipitated with an anti-C-terminal $p34^{cdc2}$ peptide antibody (Fig. 3*b*, lanes 4, 8 and 9). The faint band just above the 33K protein in the anti-cyclin A immunoprecipitate in lane 8 could be $p34^{cdc2}$ as it comigrates with the fastest migrating (least phosphorylated) form of $p34^{cdc2}$, but there was too little material to analyse further. The 33K protein is strongly labelled with ^{32}P *in vivo* (Fig. 3*c*), arguing against its being $p34^{cdc2}$ because the highly phosphorylated form of $p34^{cdc2}$ migrates at a position corresponding to ~35K. When the 33K protein is released from a ^{35}S-labelled anti-cyclin A immunoprecipitate it can be reprecipitated with an anti-PSTAIR peptide antibody, but not with an anti-C-terminal $p34^{cdc2}$ peptide antibody (Fig. 3*d*). Tryptic peptide mapping confirms that this protein is distinct from the $p34^{cdc2}$ detected in cyclin B immunoprecipitates (data not shown). Consistent with our conclusion that cyclin A does not primarily associate with $p34^{cdc2}$, we do not detect a 60K protein comigrating with cyclin A in immune complexes made with the anti-C-terminal $p34^{cdc2}$ peptide antibody (Fig. 3*b*, lane 4). Interestingly, we do detect a 60K protein with an identical tryptic peptide map to cyclin A bound to $p13^{suc1}$-Sepharose (Fig. 3*b*, lane 5), which suggests that $p13^{suc1}$ binds both $p34^{cdc2}$ and closely related proteins, such as the 33K protein.

Despite the apparent absence of $p34^{cdc2}$ in cyclin A immunoprecipitates, these immunocomplexes have histone H1 kinase activity *in vitro* (Fig. 4), but differ from those of cyclin B. Cyclin B-associated H1 kinase activity increases dramatically as the cells enter mitosis. In contrast, the cyclin A-associated H1 kinase activity increases only slightly and gradually through S and G2 phase, roughly paralleling the rise in cyclin A protein, and reaches a maximum before that of cyclin B (Fig. 4). The cyclin A-associated H1 kinase activity begins to decrease before that of cyclin B, consistent with the earlier decline in cyclin A protein. As in clams[16], human cyclin B is stable in cells arrested with microtubule inhibitors such as nocodazole, but cyclin A is not (data not shown). This results in an artefactually low level of cyclin A-associated histone H1 kinase activity in nocodazole-arrested cells and explains why this complex was proposed to be the interphase form of $p34^{cdc2}$ (ref. 12).

At first sight our results seem to conflict with the finding in clams that cyclin A associates with $p34^{cdc2}$ (ref. 8). But in clams cyclin A is not reliably coprecipitated with anti-$p34^{cdc2}$ antibodies, although it is easily detected using $p13^{suc1}$-Sepharose. In studies on HeLa cells Giordano *et al.*[12] found that p60 could be immunoprecipitated by anti-cdc2 C-terminal antibody, but the level of p60 was considerably less than that of cyclin B. So far we have been unable to detect cyclin A-associated $p34^{cdc2}$ by immunoblotting the anti-cyclin A immunoprecipitates with an anti-C-terminal $p34^{cdc2}$ peptide antibody (data not shown), but it remains possible that cyclin A binds to a minor fraction of the underphosphorylated form of $p34^{cdc2}$.

In summary, we have shown that human cyclin A mRNA and protein levels are regulated in the cell cycle and rise and fall in advance of those of cyclin B. The close but staggered induction of cyclin A and B mRNA transcription in the cell cycle shows that this sytem is tightly regulated at the nuclear level. There are several differences at the protein level between cyclin A and cyclin B, notably in the proteins with which they associate and the timing of their associated histone H1 kinase activities. Cyclin B associates with $p34^{cdc2}$ to form a complex in G2 phase, but this does not become fully active as a protein kinase until the $p34^{cdc2}$ subunit is dephosphorylated on phosphotyrosine and

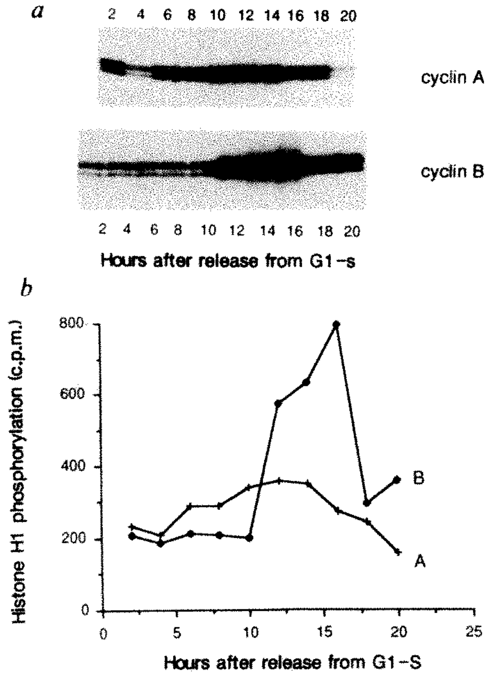


FIG. 4 *In vitro* kinase activities associated with cyclin A and B vary during the cell cycle. *a*, Kinase assay of cyclin A or B immunoprecipitates with histone H1 as a substrate. Cells were synchronized by a thymidine-aphidicolin block and samples taken at the indicated times after release from the block. The autoradiograph was exposed without a screen for 14 h at room temperature. Flow cytometry analysis showed the G2–M peak to be at 14 h. *b*, Incorporation of label into histone H1 from the gel in panel A estimated by a beta scanner.

METHODS. For kinase assays, 1×10^6 cells were lysed in RIPA buffer per time point and H1 kinase assays and cyclin B immunoprecipitations were prepared and performed as previously described[11]. For cyclin A immunoprecipitations, 10 μl monoclonal antibody C160 cell supernatant was used per 100 μg cell lysate. The same lysates were used for the anti-cyclin A and the anti-cyclin B immunoprecipitations.

phosphothreonine in late G2 (refs 18, 19). In contrast, cyclin A associates with a 33K protein related to $p34^{cdc2}$ and this complex does not show a dramatic increase in *in vitro* kinase activity as cells enter M phase, but rather its kinase activity gradually increases as cells progress from S to G2 phase. Although we have not demonstrated that the 33K protein found in anti-cyclin A immunoprecipitates is a protein kinase, this seems likely from its close relation to $p34^{cdc2}$; not only does the 33K protein contain the PSTAIR motif, but it also has a very similar fragmentation pattern to $p34^{cdc2}$ when cleaved with *N*-chlorosuccinimide[12]. (There are now several reports of cdc2-related proteins that have the PSTAIR motif in other species, notably *Xenopus*, *Drosophila* and *Saccaromyces cerevisiae*[20], which all have four conserved tryptophans, the residue recognized by *N*-chlorosuccinimide). In addition, cyclin A itself is not likely to be a protein kinase as its sequence does not have any of the conserved motifs found in all known protein kinases. So far few differences have been noted in the protein kinase specificities of the two types of cyclin complex *in vitro*[20], which also implies that the cyclin A-associated kinase is very similar to $p34^{cdc2}$. Like the form of $p34^{cdc2}$ associated with cyclin B, the 33K protein associated with cyclin A contains phosphotyrosine (data not shown). Thus, by analogy with $p34^{cdc2}$, the protein kinase activity of the cyclin A complex might also be regulated by phosphorylation of its catalytic subunit.

As cyclin A complexes have protein kinase activity earlier in the cell cycle than the cyclin B-$p34^{cdc2}$ complex, it is possible that the cyclin A complex is involved in the S phase itself, fulfilling the role in human cells performed by the 'G1 cyclins' in *S. cerevisiae*[21]. However, this seems unlikely as we find that human cyclin A mRNA causes *Xenopus* oocyte maturation, which implicates it in the induction of mitosis.

It is intriguing that cyclin A has now been indirectly linked with cellular transformation in two ways. First, p60 was initially described through its association with E1A in adenovirus-transformed cells[22]. E1A binds to and probably inactivates the retinoblastoma protein[23,24], and in an analogous fashion may also modulate the function of cyclin A. Second, hepatitis B virus sequences were found integrated into an intron of the cyclin A gene in a hepatocellular carcinoma[15]. Thus it is possible that disrupting the normal function of cyclin A has profound effects on the regulation of the cell cycle. The nature of this disruption and whether the same is also true for cyclin B now need to be investigated. ☐

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Sequential activation of *HOX2* homeobox genes by retinoic acid in human embryonal carcinoma cells

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RETINOIC acid has been implicated as a natural morphogen in chicken[1–4] and frog[5] embryogenesis, and is presumed to act through the gene regulatory activity of a family of nuclear receptors[6–10]. Homeobox genes, which specify positional information in *Drosophila* and possibly in vertebrate embryogenesis[11–21], are among the candidate responsive genes[4,5,14]. We previously reported that retinoic acid specifically induces human homeobox gene (*HOX*) expression in the embryonal carcinoma cell line NT2/D1 (ref. 22). We now show that the nine genes of the *HOX2* cluster are differentially activated in NT2/D1 cells exposed to retinoic acid concentrations ranging from 10^{-8} to 10^{-5} M. Genes located in the 3′ half of the cluster are induced at peak levels by 10^{-8} M retinoic acid, whereas a concentration of 10^{-6} to 10^{-5} M is required to fully activate 5′ genes. At both high and low retinoic acid concentrations, *HOX2* genes are sequentially activated in embryonal carcinoma cells in the 3′ to 5′ direction.

The cloned human embryonal carcinoma (EC) cell line NT2/D1 shows the phenotype of multipotent embryonic stem cells, and is induced to differentiate by retinoic acid (RA) into a variety of cell types, including neurons[23]. Cells were cultured for 14 days in medium containing RA at different concentrations in the non-toxic range of 10^{-8} to 10^{-5} M. Differentiation was monitored by the proportion of cells positive to glycolipid surface markers expressed in either stem or differentiated EC cells[23]. An RA concentration of 5×10^{-8} M was sufficient to cause disappearance of ~50% stem cells and significant expression of differentiation antigens (Fig. 1*a*). Total RNA was extracted from 14-day cultures and probed for expression of *HOX2* genes. Maximal levels of transcript accumulation from genes located in the 5′ half of the *HOX2* cluster (that is, from *HOX2E* to *HOX2A*) occurred in cells exposed to at least 5×10^{-6} M RA, whereas decreasing RNA levels were detected in cells exposed to lower RA concentrations (Fig. 1*b*). Conversely, transcripts from the 3′ genes *HOX2F*, *HOX2H* and *HOX2I* were accumulated at peak levels at RA concentrations of around 10^{-8} M. Thus, *HOX2* genes are differentially activated by RA in EC cells, that is, 3′ genes are activated at maximum levels by RA concentrations that are two orders of magnitude lower than those required to fully activate 5′ genes. *HOX2A* apparently marks the border between the two groups. An apparent exception is represented by *HOX2G*, which despite being located in the 3′ group showed an activation pattern more typical of the 5′ group (Fig. 1*b*).

The number and size of *HOX2* gene transcripts observed in RA-induced EC cells were virtually identical to those observed in human embryos[15,17,20]. We also analysed the expression pattern of *HOX4B*, which is expressed in human embryos in several transcripts according to a spatially restricted and stage-specific pattern[16]. Northern blot analysis indicated that a 4.2-kilobase (kb) transcript accumulated in NT2/D1 cells after exposure to

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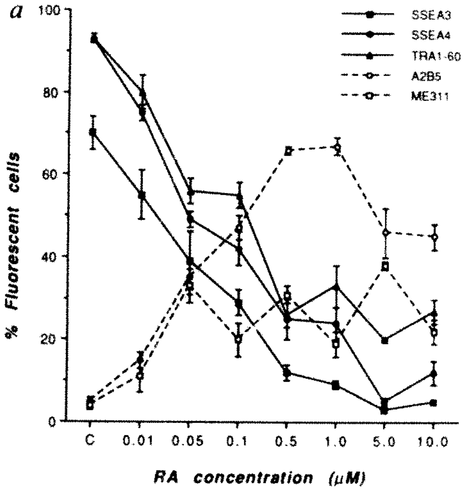
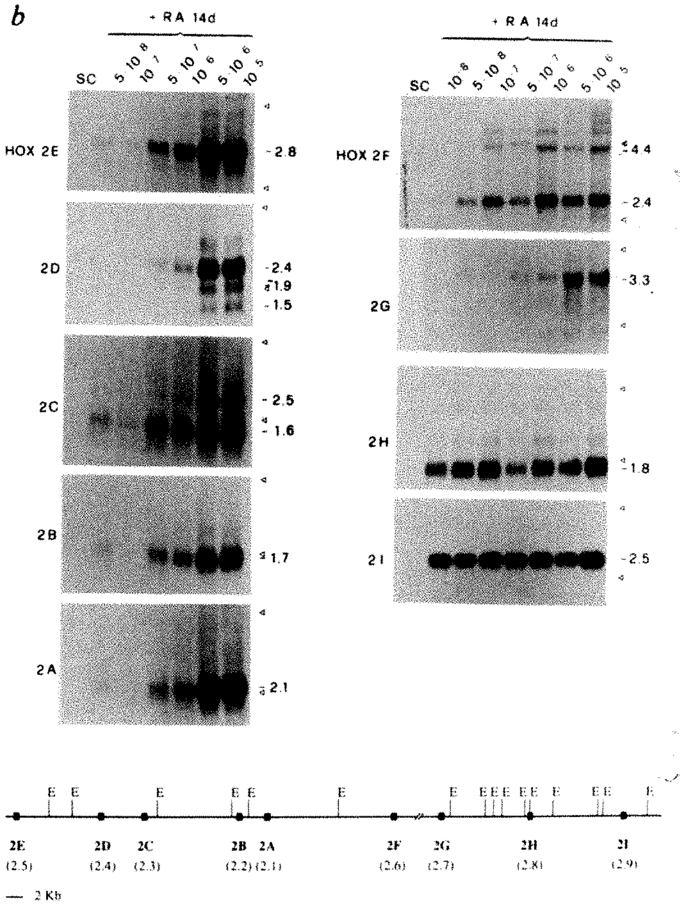
10^{-8} M RA, whereas 5.4-kb and 2.8-kb transcripts became increasingly abundant starting from 10^{-6} M RA (Fig. 1*c*). The anterior border of expression of the 4.2-kb transcript in 6–7-week human embryonic central nervous system (CNS) is in the hindbrain region, whereas 5.4- and 2.8-kb transcripts are expressed more caudally in the spinal cord, as well as in the limb bud[16]. The present data indicate that different RA concentrations direct alternative expression patterns from *HOX4B* in EC cells, reproducing a region-specific developmental regulation observed *in vivo*.

We then studied the time course of the RA-induced *HOX2* gene activation in conditions in which all *HOX2* genes, or only the 3′ genes are activated, that is, in the presence of 10^{-5} M or 10^{-7} M RA respectively. Expression of single genes was analysed by RNase protection assay. At 10^{-5} M RA, transcripts from *HOX2H* and *HOX2I* rapidly and steadily accumulated, reaching peak levels at 24 h of RA exposure. Activation of the other genes proceeded sequentially from *HOX2G to HOX2E* between 1 and 4 days of RA induction (Fig. 2*a*). Fifty per cent maximal RNA accumulation levels were reached at 14 h for *HOX2I* and *HOX2H*, 60 h for *HOX2G*, *HOX2F* and *HOX2A*, 70 h for *HOX2B*, 90 h for *HOX2C*, 115 h for *HOX2D* and 180 h for *HOX2E* (Fig. 2*b*). The *HOX2* cluster is thus progressively activated in the 3′ to 5′ direction, according to a discrete pattern. As previously reported, continuous presence of RA is required throughout the induction process to activate 5′ genes[22].





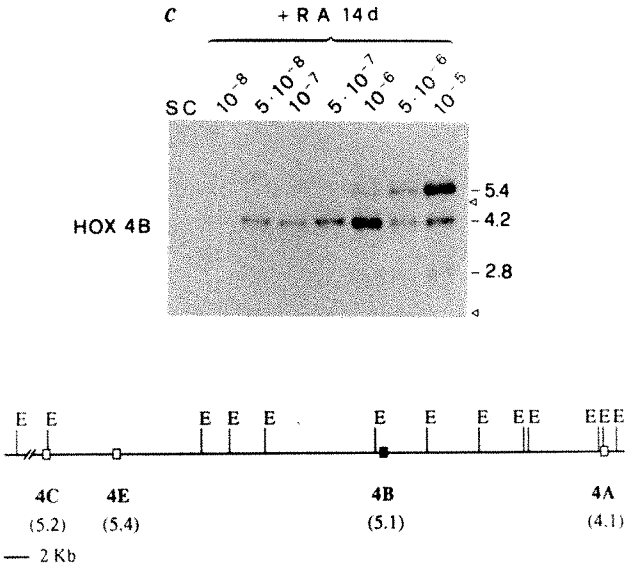
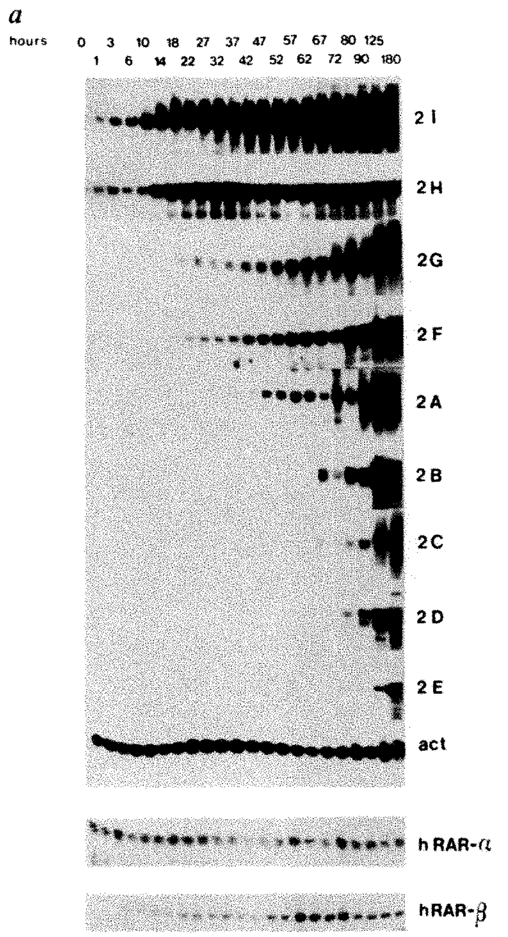


FIG. 1 *a*, Expression of glycolipid surface antigen markers in the human EC cell line NT2/D1, before (C) and after induction with RA, at concentrations ranging 10^{-8}–10^{-5} M. The EC stem cell markers SSEA-3, SSEA-4 and TRA1-60, and two antigens, ME311 and A2B5, which are expressed in EC-derived differentiated cells, were analysed using the corresponding monoclonal antibodies[22]. Neurofilament-positive neurons are observed among the A2B5$^+$, ME311$^-$ cell population. Mean ± s.e.m. of 3–6 different experiments are shown. *b*, Northern blot analysis of expression of *HOX2* genes and *c*, the *HOX4B* gene in NT2/D1, before (SC) and after 14 days of induction with 10^{-8}–10^{-5} M RA. Transcript sizes are in kb. Open triangles, mobilities of 28S and 18S ribosomal RNAs used as size markers. Partial restriction maps of the respective clusters are shown at the bottom of each panel. *HOX* genes are identified according to the nomenclature for human homeobox genes[26]. Related mouse designations indicated in parentheses. Black boxes, homeoboxes studied. E, *Eco*R1. Breaks shown in the *HOX2* and *HOX4* maps are 18 and 15 kb respectively.

METHODS. The cloned, human EC cell line NT2/D1 (ref. 23) was maintained in high-glucose DMEM supplemented with 10% FCS in a humidified atmosphere of 5% CO_2 in air. Differentiation was induced by seeding cells at 5×10^5 per 75 cm^2 flask in 10^{-8}–10^{-5} M RA (all-*trans*, Sigma Chemical Co.). Cells were refed every 4 days with RA-containing medium and analysed after 14 days by indirect immunofluorescence, as previously described[22,23]. Total RNA was extracted by the guanidine-isothiocyanate technique[27], selected for poly(A)$^+$ by one passage on oligo(dT) cellulose columns according to standard procedures, run in 2–3 μg aliquots on 1.0% agarose-formaldehyde gel, transferred by northern capillary blot[28] onto nylon membranes (Hybond-N, Amersham) and hybridized to 10^7 c.p.m. DNA probe labelled by random priming to a specific activity of 10^9 d.p.m. μg^{-1}. Filters were washed under stringent conditions (0.1 × SSC buffer, 0.2% sodium dodecyl sulphate (SDS) at 65 °C for 60 min) and exposed to Kodak XAR-5 film at −70 °C for 1–3 days. Filters were stripped and reprobed up to six times as previously described[22]. *HOX2* probes were: a 3.0-kb *Sma*I genomic fragment of *HOX2E*; a 1.5-kb *Eco*R1-*Bgl*II fragment of *HOX2D*; a 1.3-kb cDNA clone (HHO.c1.95) from *HOX2C* (ref. 17); a 2.0-kb *Eco*R1 fragment of *HOX2B*; a 1.2-kb cDNA clone (HHO.c10) from *HOX2A* (ref. 15); a 0.3-kb *Hin*dIII fragment of *HOX2F*; a 0.8-kb *Bgl*II-*Eco*R1 fragment of *HOX2G*; a 2.8-kb *Eco*R1 fragment of *HOX2H* and a 0.6-kb *Pvu*II fragment of *HOX2I*. The *HOX4B* probe was a 1.3-kb cDNA clone (HHO.c13)[16].



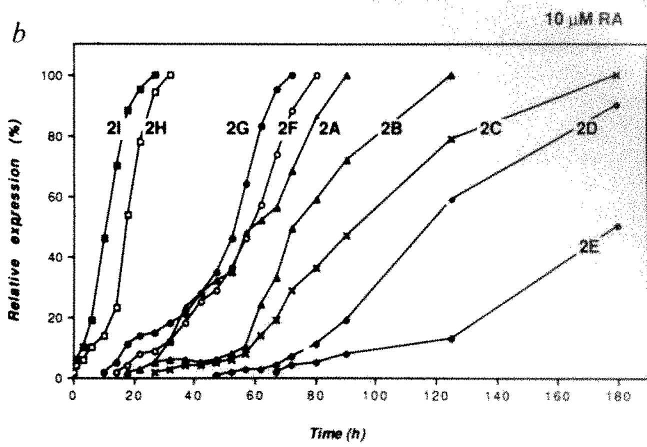
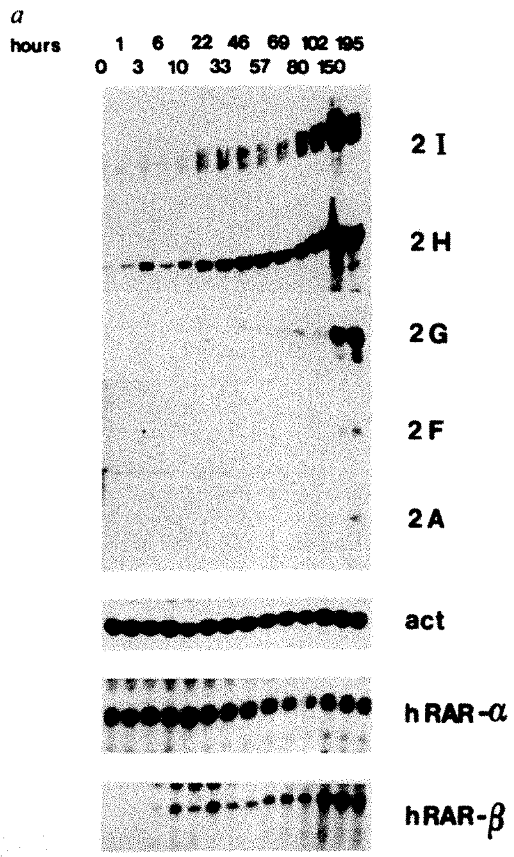


FIG. 2 *a*, RNase protection analysis of expression of the nine *HOX2* genes and human α- and β-retinoic acid receptor genes (hRAR-α, hRAR-β) in total RNA from NT2/D1 cells, uninduced (0) and induced with 10^{-5} M RA for 1–195 h. Expression of β-actin is an internal control. *b*, Values obtained for *HOX2* genes by densitometric scanning of autoradiographs, expressed in arbitrary units; 100 represents plateau RNA accumulation levels. These levels were comparable among different genes.

METHODS. RNA was obtained as described in the legend to Fig. 1. Partial 3′ noncoding sequences from *HOX2* genes and from the human β-actin gene[29] were subcloned in pGEM vectors (Promega Biotec). A *Sma*I–*Eco*R1 fragment from hRAR-α and an *Eco*R1–*Bam*HI fragment from hRAR-β cDNAs[6,8] were subcloned in pBluescript vectors (Stratagene). Antisense strand RNA probes were synthesized with Sp6 or T7 polymerase and hybridized to 20 μg RNA at appropriate temperatures. RNase digestion and electrophoresis on 7% urea–polyacrylamide gels were carried out as described previously[30].



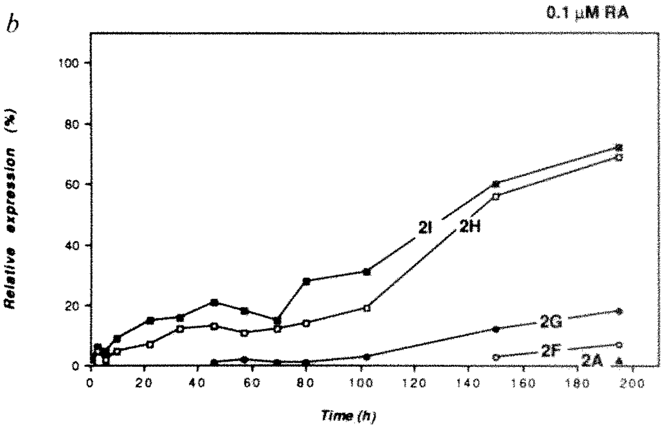


FIG. 3 *a*, RNAse protection analysis of expression of *HOX2I, 2H, 2G, 2F, 2A*, hRAR-α, hRAR-β and β-actin genes in total RNA from NT2/D1 cells, before (0) and after induction with 10^{-7} M RA for 1–195 h. Expression of *HOX2B, 2C, 2D and 2E* was undetectable up to 195 h (not shown). Values from autoradiograph densitometric scanning are shown in *b* (see legend to Fig. 2). Methods as for Fig. 2.

The time course of *HOX2* gene activation in cells induced by 10^{-7} M RA is shown in Fig. 3. Genes from *HOX2I* to *HOX2A* were activated in the same order observed in the presence of 10^{-5} M RA, although with a slower kinetics. Transcripts from genes upstream of *HOX2A* were undetectable throughout the period examined. It is important to note that NT2/D1 cells are morphologically and phenotypically homogeneous in the first week of culture, with no obvious difference between low and high RA levels[23]. But we cannot rule out the possibility that 5′

genes, which are activated only at high RA levels, may be responding secondarily to differentiation events.

We also studied the expression of different RA receptors in NT2/D1 cells. Northern blot analysis showed transcripts for the α- and γ-receptors (hRAR-α and hRAR-γ) in both untreated and RA-treated EC cells, whereas hRAR-β transcripts were abundantly accumulated only in RA-treated cells, with the whole range of RA concentrations (data not shown). Appearance of hRAR-β transcripts slightly followed that of the early responders *HOX2I* and *HOX2H* (Figs 2*a* and 3*a*).

The level of RA needed to fully activate 5′ *HOX2* genes in EC cells, 5×10^{-6} M) appears to be higher than the levels reported in chick limb buds (10^{-8}–10^{-7} M) (ref. 2) or *Xenopus* embryos (1.5×10^{-7} M) (ref. 5), although there is evidence that RA concentrations of the order of 10^{-6} M may be physiological in mammals[24]. To estimate the range of effective RA concentrations in our experimental system, we cotransfected NT2 cells with an expression vector for human RAR-α (hRAR-α0) and a reporter plasmid $(\text{TRE3})_3$-tk-CAT containing a synthetic RA-responsive element[9]. Transfected cells were exposed to increasing RA concentrations and RA-induced activation was estimated by determining chloramphenicol acetyltransferase (CAT) activity expressed from the reporter gene (Fig. 4). Transactivation was observed starting from 10^{-10} M RA and steadily increased up to 10^{-5} M, with an estimated 50% maximum inducible CAT activity around values of 10^{-7} M. The range of effective concentrations in NT2 cells appears to be wider than previously observed in HeLa cells[6,8,9]. The high levels of cytoplasmic RA-binding protein typical of human teratoma-derived cells[25] might at least in part account for this difference. A comparison between RA levels effective on cell lines *in vitro* and putative physiological values *in vivo* (as yet unknown in mammals) seems premature, and must await collection of more data *in vivo*.

We have shown that human *HOX2* genes are differentially activated in EC cells by RA in a concentration-dependent fashion and in a sequential order which is colinear with their 3′ to 5′ arrangement in the cluster. The same polarity is observed in the expression pattern of *HOX2* genes along the antero-posterior axis of the developing human and mouse CNS, where 3′ genes are expressed more rostrally in myelencephalon and 5′ genes more caudally in the spinal cord[13,19-21]. The present data suggest that temporal and/or spatial modulation of RA levels might, at least in part, underlie the observed spatially restricted *HOX2* gene expression patterns. This could in turn provide positional information required for further developmental decisions. It is interesting to note that *HOX2A*, which marks the border between genes requiring high or low levels of RA for their activation *in vitro*, is the homologue of mouse *Hox-2.1*, which marks the border between hindbrain and spinal cord *in vivo*[19]. □

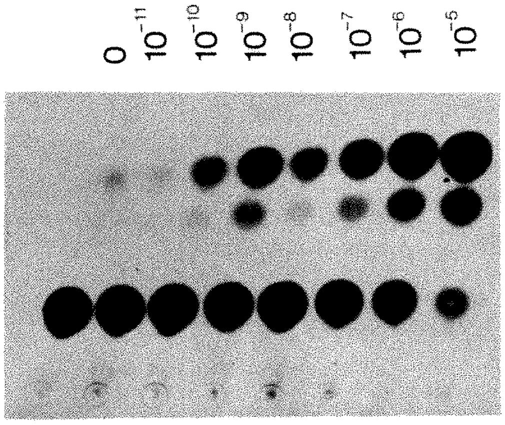


FIG. 4 RA-dependent transcriptional transactivation by hRAR-α in NT2/D1 cells. An expression vector containing the human RAR-α cDNA under the control of the simian virus 40 (SV40) early promoter (hRAR-α0) was cotransfected with a reporter plasmid, $(\text{TRE3})_3$-tk-CAT, containing a synthetic RA responsive element[9]. Cells were then exposed to increasing RA concentrations (10^{-11}–10^{-5} M as shown) and assayed for transient CAT activity.

METHODS. NT2/D1 cells were cotransfected with hRAR-α0 (5 µg) and $(\text{TRE3})_3$-tk-CAT (15 µg), by a standard calcium phosphate technique[31]. After transfection (24 h) cells were fed with media containing different RA concentrations (10^{-11}–10^{-5} M) or no RA (0). After 48 h of culture in RA-containing medium, cells were collected and cytosolic extracts prepared for CAT activity determination by TLC according to standard techniques[31].

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Reversible inactivation of K^+ channels of *Vicia* stomatal guard cells following the photolysis of caged inositol 1,4,5-trisphosphate

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RECENT investigations suggest that cytoplasmic D-*myo*-inositol 1,4,5-trisphosphate (InsP_3) functions as a second messenger in plants, as in animals, coupling environmental and other stimuli to intracellular Ca^{2+} release[1,2]. Cytoplasmic levels of InsP_3 and the turnover of several probable precursors in plants are affected by physiological stimuli—including light, osmotic stress and the phytohormone indoleacetic acid[3-5]—and InsP_3 activates Ca^{2+} channels[6] and Ca^{2+} flux across plant vacuolar[7] and microsomal membranes[8]. Complementary data also link changes in cytoplasmic free Ca^{2+} to several physiological responses, notably in guard cells which regulate gas exchange through the stomatal pores of higher plant leaves. Recent evidence indicates that guard cell K^+ channels and, hence, K^+ flux for stomatal movements[9] may be

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controlled by cytoplasmic Ca^{2+} (ref. 10). So far, however, direct evidence of a role for $InsP_3$ in signalling in plants has remained elusive. Here we report that $InsP_3$ released from an inactive, photolabile precursor, the P^5-1-(2-nitrophenyl)ethyl ester of $InsP_3$ (caged $InsP_3$)[11] reversibly inactivates K^+ channels thought to mediate K^+ uptake by guard cells from *Vicia faba* L. while simultaneously activating an apparently time-independent, inward current to depolarize the membrane potential and promote K^+ efflux through a second class of K^+ channels[12,13]. The data are consistent with a transient rise in cytoplasmic free Ca^{2+} (ref. 9) and demonstrate that intact guard cells are competent to use $InsP_3$ in signal cascades controlling ion flux through K^+ channels.

Caged $InsP_3$ was biologically inactive in the guard cells, as could be determined electrically. Bathed in 5 mM Ca^{2+}-HEPES buffer, pH 7.4, with 0.1 mM KCl, intact guard cells showed membrane potentials and resistances of -137 ± 4 mV and 12 ± 1 kΩ cm^2 ($n = 11$) when impaled with microelectrodes containing caged-$InsP_3$ (final concentration, 0.15 mM in 70 mM K^+-acetate, pH 7.5). Equivalent results were obtained with microelectrodes filled with K^+-acetate plus 0.15 mM caged ATP (the P^3-1-(2-nitrophenyl)ethyl ester of ATP) or with K^+-acetate alone, and in all cases the steady-state current-voltage (I-V) characteristics were dominated by an outward-rectifying K^+ channel current at positive-going voltages[12].

Raising the K^+ concentration of the bathing medium (K_o^+) to 30 mM depolarized the cells to -33 ± 2 mV ($n = 9$) and exposed a time-dependent, inward-going current which activated at voltages negative of -100 mV (Fig. 1*a*, *b*). The current was reversibly blocked by the K^+ channel antagonists tetraethylammonium chloride (30 mM) and 10 mM $BaCl_2$ (data not shown), and analyses of tail currents (Fig. 1*c*) revealed a Nernstian dependence on K_o^+ over the concentration range 3-30 mM (Fig. 1*d*), indicating that this inward-rectifying current is carried by K^+.

Light flashes on cells loaded with caged $InsP_3$ were followed by membrane depolarizations ($+15 \pm 2$ mV, $n = 16$) which subsequently recovered. Voltage trajectories varied somewhat from cell to cell (see Figs 2-4); in all but three trials, however, depolarizations were complete within 2 min (mean ± s.e., 0.9 ± 0.3 min). Figure 2 shows that, underlying this voltage response, the effect was to suppress the time- and voltage-dependent inward (K^+ channel) current while increasing the instantaneous (leak) current. By contrast, the outward-rectifying K^+ current showed no measurable response in seven cells; in four cells it was reduced in scalar fashion but recovered within 2-3 min (compare Fig. 3).

Figure 3 indicates a voltage-dependence to the response of the inward-rectifying K^+ current. Flash-evoked inactivation of the current was marked both by a decline in the current magnitude, and by a shift of around -50 mV in the voltage at which 50% activation occurred (Fig. 3*b*). These same characteristics are evident in Fig. 4 which shows that a graded response of the inward-rectifier could be achieved with increasing $InsP_3$ release (flash intensity). Figure 4 also shows that raising the cytoplasmic Ca^{2+}-buffering capacity, by including 20 mM EGTA in the microelectrode, was antagonistic to the $InsP_3$-evoked response (compare Fig. 4*c* and *d*).

Recovery of the K^+ channel and leak currents—and of membrane voltage—was correlated with flash exposure. Focusing flashes on impaled cells using the camera port of the microscope resulted in current and voltage responses which recovered fully over 5-8 min (Fig. 3); higher beam intensities could be achieved by mounting the flashlamp beside the microscope and directing the beam on the chamber, but with the disadvantage that the caged compound in the microelectrode was exposed also;

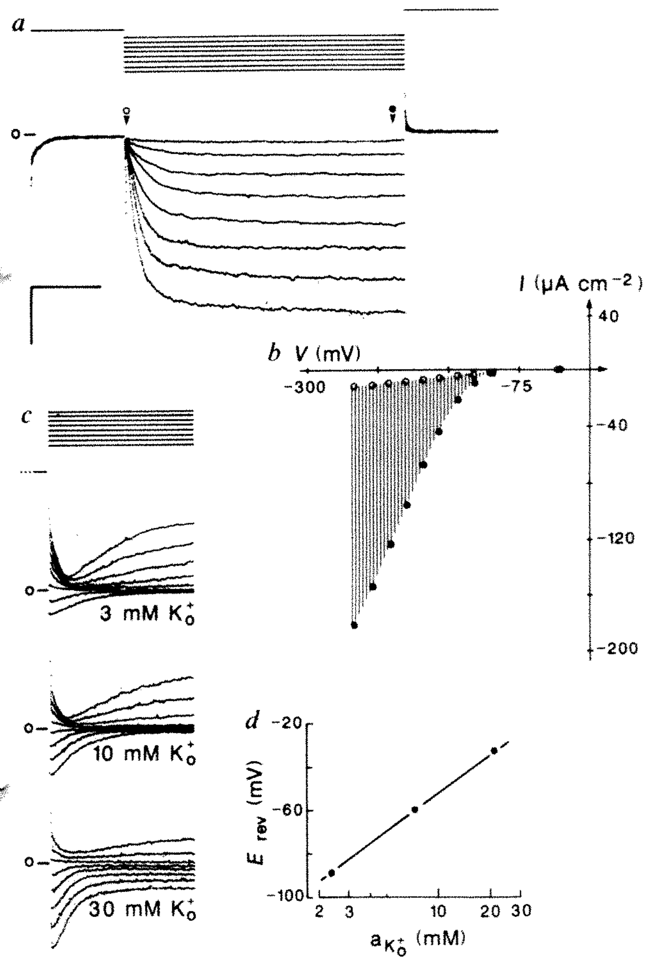

FIG. 1 Inward-rectifying K^+ channel current (I) of intact *Vicia* guard cells. Data from one cell: surface area, 1.6×10^{-5} cm^2; volume, 3.4 pl; stomatal aperture, 6 μm. *a*, Kinetics of voltage-dependent activation in 30 mM K_o^+. Eight cycles of three voltage-clamp steps (above): conditioning voltage, -100 mV; test voltages, -120 to -250 mV; tailing voltage, -30 mV. Current zeros indicated to the left. Scale bar: vertical, 60 μA cm^{-2} or 200 mV; horizontal, 500 ms. *b*, Current–voltage characteristics of the instantaneous (○, leak) and steady-state (●; K^+ channel plus leak) currents from traces in *a* (sampling times cross-referenced by symbol; steady-state current at -100 mV included). Shaded difference is the K^+ current. *c*, Current 'tails' recorded after 1 s conditioning steps to -250 mV. Above, clamp voltage steps (8; 0 to -120 mV); below, tail currents recorded in 3, 10 and 30 mM K_o^+. Current zeros indicated to the left. Scale bar: vertical, 40 μA cm^{-2} or 200 mV; horizontal, 200 ms. The late rising currents at more positive voltages reflect activation of the outward-rectifying K^+ current[12]. *d*, Dependence of tail current reversal potential (E_{rev}) on extracellular K^+ activity ($a_{K_o^+}$). E_{rev} taken from current relaxations in *c* and K_o^+ corrected for activities in free solution (see ref. 12). Slope of the line, +59 mV/$a_{K_o^+}$ decade.

METHODS. *Vicia faba* L. was grown and epidermal peels containing the guard cells were mounted as described previously[13,20]. Peels were bathed in fast-flowing solutions containing KCl and 5 mM Ca^{2+}-HEPES buffer, pH 7.4, or 5 mM Ca^{2+}-MES, pH 6.1[12,14]. Electrical recordings were carried out using double-barrelled microelectrodes[20] filled with 200 mM K^+-acetate[12,14]. Surface areas were calculated from the orthogonal dimensions of impaled cells[12,14]. Cells were loaded with the caged compounds, inositol phosphates and Ca^{2+} buffers by diffusion from the microelectrode (voltage-recording barrel) and the K^+-acetate filling solution was doped accordingly. Estimates of loading times based on calculated and measured electrolyte leakage rates from microelectrodes[14,21] indicated that the compounds should equilibrate with the cell cytoplasm within 15–20 min; trials with the caged compounds generally were begun 15 min, and in some experiments with caged ATP, 25–30 min after impalement. Caged $InsP_3$ and caged ATP were photolysed by 1-ms flashes from a xenon flashlamp (Chadwick Helmuth, El Monte, USA). Photochemical release was calibrated in trials with 5 μl caged ATP placed in the experimental chamber and the ATP released was measured by HPLC[22] on a Parasil 10SAX column with a NaH_2PO_4 gradient. A single, full-intensity flash (55 mJ, effective wavelengths 300–350 nm recorded through a UG11 filter) released 9% of the caged compound and this value was used also in estimating $InsP_3$ release[11]. Because of uncertainties about diffusional loading from the microelectrode[21], however, the estimates should be treated as maxima only.

recoveries in these instances were slow (10-20 min) or incomplete (Figs 2, 4), probably a consequence of InsP$_3$ release in the microelectrodes and its subsequent diffusion into the cells.

The flash-dependent effects on K$^+$ channel and leak currents were specifically associated with the release of InsP$_3$. Both the current and voltage responses were reproducible and could be repeated in any one guard cell impaled with a microelectrode containing caged InsP$_3$; comparable electrical characteristics were obtained with microelectrodes containing 10 μM native InsP$_3$ ($n = 4$) but not with D-*myo*-inositol 1,4-bisphosphate ($n = 3$). Furthermore, light flashes failed to evoke any response in cells ($n = 13$) impaled with microelectrodes containing 0.15 mM caged-ATP. The latter observation provides strong evidence also that neither the light flash itself, nor the by-products from photolysis of caged InsP$_3$ (2-nitrosoacetophenone and H$^+$ (ref. 11)) had any short-term or lasting effect on the cells.

We note that a physiological effect of InsP$_3$ on transport across the plasma membrane is easily explained only if caged InsP$_3$ were loaded in the cytoplasm, that is, if, in each case, the microelectrode tip was located in the cytoplasmic compartment. This interpretation accords with the results of dye injections[10] and Cl$^-$ leakage from microelectrodes[14] into *Vicia* guard cells, and with reports of cytoplasmic pH and pCa values recorded with ion-sensitive microelectrodes[15,16]. We conclude, as have others[10], that physiologically viable impalements of higher plant cells—in our hands, those that yield stable electrical characteristics over many tens of minutes—leave the microelectrode tip in the cytoplasm.

The primary response to InsP$_3$ release in these experiments was most probably a rise in cytoplasmic free Ca^{2+}. Estimates of the jump in InsP$_3$ generated by a flash (see legend to Fig. 1) lie in the range 0.5-10 μM, concentrations effective in releasing Ca^{2+} from plant vacuoles[17]. Furthermore, the effects of InsP$_3$ release were countered by raising the cytoplasmic Ca^{2+}-buffering capacity, although channel activity in the control appeared unaffected (Fig. 4*d*). There is, also, a remarkable similarity between our data and those reported for *Vicia* guard cell protoplasts loaded with Ca^{2+} (ref. 9): in each case, the effect was to reduce inward-rectifying K$^+$ currents and alter channel gating,

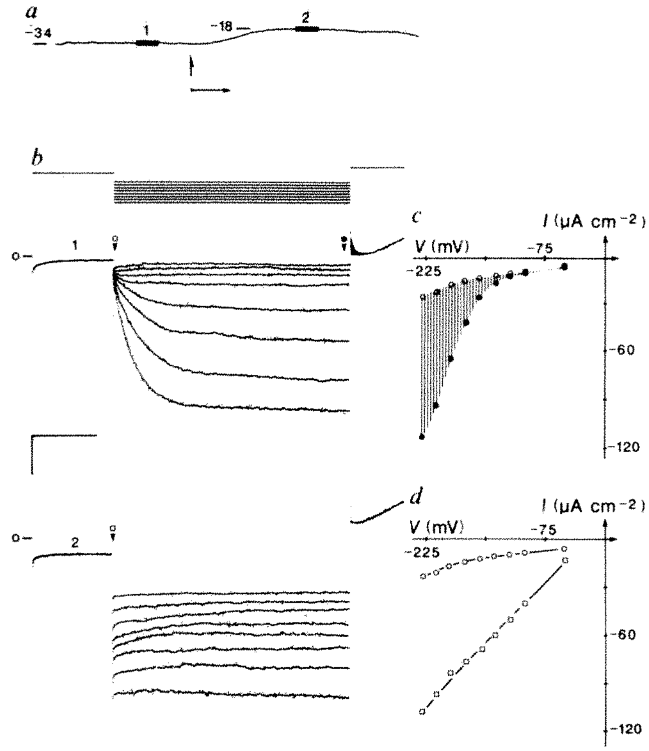

FIG. 2 K$^+$ channel and leak current response to photolytic release of InsP$_3$ from caged InsP$_3$. Data from one *Vicia* guard cell loaded with 0.15 mM caged InsP$_3$ and bathed in 5 mM Ca^{2+}-MES, pH 6.1, with 30 mM KCl. Cell parameters: surface area, 1.6×10^{-5} cm^2; volume, 4.1 pl; stomatal aperture, 7 μm. *a*, Free-running membrane potential (values in mV) before and after flash (↑, 25 mJ; estimated InsP$_3$ release, 5 μM). Voltage clamp cycles (masked from trace) were run during the barred periods. Timescale, 1 min. *b*, Current trajectories recorded during the periods (1, before; 2, after flash) indicated in *a*. Current zeros indicated to left. Clamp cycle (above): conditioning voltage, −50 mV; test voltages (8), −100 to −250 mV; tailing voltage, 0 mV. Scale bar: vertical, 30 μA cm^{-2} or 300 mV; horizontal, 300 ms. Instantaneous (○, □) and stead-state (●) currents taken at times indicated (cross-referenced to *c*, *d* by symbol). Note the absence of the time-dependent (K$^+$) current after the flash. *c*, Current-voltage relations for the instantaneous (○; leak) and steady-state (●; K$^+$ channel plus leak) currents before the flash (steady-state current at −50 mV included). Shading indicates the time-dependent (K$^+$ channel) component. *d*, Instantaneous current-voltage relations before (○) and after (□) flash, showing around fivefold rise in leak conductance.

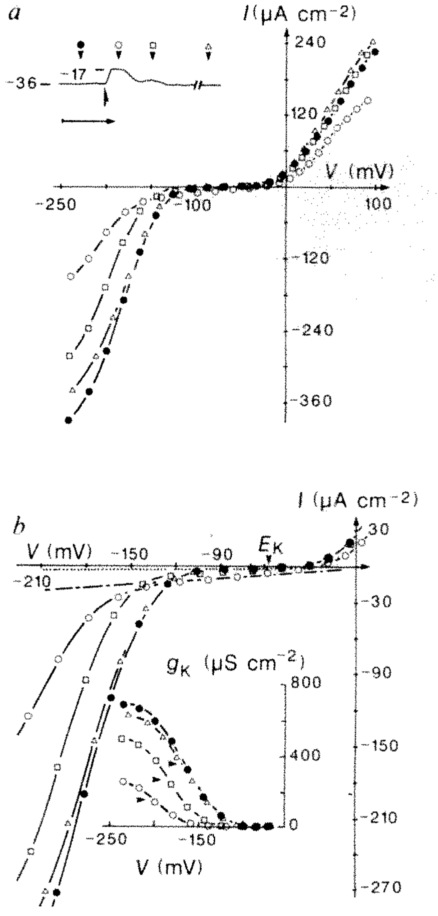

FIG. 3 Time course for K$^+$ channel and leak current response and recovery following photolytic release of InsP$_3$. Data from one *Vicia* guard cell loaded with 0.15 mM caged InsP$_3$ and bathed in 5 mM Ca^{2+}-MES, pH 6.1, with 10 mM KCl. Cell parameters: surface area, 1.6×10^{-5} cm^2; volume, 3.6 pl; aperture 8 μm. *a*, Current-voltage curves determined before (●) and after (○, □, △) flash through microscope camera port (↑, 5 mJ; estimated InsP$_3$ release, 1 μM). Current-voltage scans run in a bipolar staircase protocol[13,20] with 400-ms pulses. Scan duration, 16 s. Inset: Voltage trace (values in mV) with times of *I*-*V* scans (masked from trace) cross-referenced by symbol (final scan, 7.6 min after flash). Timescale, 2 min. *b*, Detail of data in *a* showing leak currents. E_K, K$^+$ equilibrium potential. The leak in the control (· · ·, 9 μS cm^{-2}) was identified from instantaneous currents (not shown) as in Fig. 1. This approach was not possible within the short response time course of cells irradiated through the microscope camera port; instead, the voltage range over which the leak and steady-state *I*-*V* scans overlapped in the control was noted and, following flashes, steady-state currents recorded over this same voltage range were used to estimate leak currents (— — —, 34 μS cm^{-2}) by linear extrapolation. Inset: Steady-state K$^+$ conductance (g_K) determined as $I_K/(V-E_K)$ after subtracting leak currents. Arrows mark 50% activation.

while promoting a time-independent, inward current. (Note that in the present circumstances, with $E_{Cl} \ll 0$ mV, a Cl^- flux[9,18] could not have accounted for this additional $InsP_3$-activated current.) Recovery following the light flashes is readily understood, by analogy with animal cell models, in context of subsequent $InsP_3$ metabolism and Ca^{2+} sequestration[1].

Finally, the results of the present study allow us to piece together independent reports on the action of abscisic acid (ABA), a phytohormone that promotes stomatal closure by inducing guard cells to lose osmotica, notably K^+ salts[19]. Exposing *Vicia* guard cells to ABA results in the rapid decline of an inward-rectifying current[13], which we now show to be carried by K^+ (Fig. 1), and a coincident rise in an unidentified leak current. Again, the behaviour parallels guard cell membrane response to Ca^{2+}. The effects may be seen to prevent K^+ uptake through the inward-rectifying channels while activating the leak to depolarize the membrane and promote K^+ loss through outward-rectifying K^+ channels[12,13]. ABA also raises the free Ca^{2+} concentration of the guard cell cytoplasm[10]. Taken together, these observations offer strong support for the action of ABA on the inward-rectifying K^+ channel and leak currents being effected by cytoplasmic Ca^{2+}.

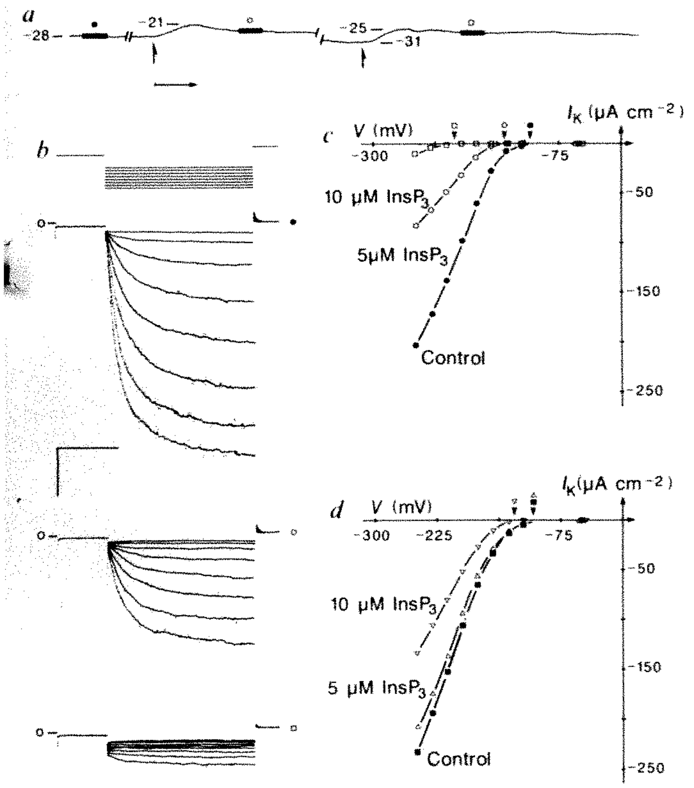

FIG. 4 Cumulative response of the K^+ current to photolytic release of $InsP_3$, and its block by buffering the cytoplasmic free Ca^{2+} concentration. Data from *Vicia* guard cells bathed in 5 mM Ca^{2+}-MES, pH 6.1, with 30 mM KCl, one cell (*a–c*) loaded with 0.15 mM caged-$InsP_3$, the second cell (*d*) loaded with 0.15 mM caged-$InsP_3$ plus 20 mM K_2 EGTA, pH 7.5 ($CaCl_2$ added to pCa ≈ 80 nM[23]). Cell parameters (both cells): surface area, 1.5×10^{-5} cm^2; volume, 3.4 pl; stomatal aperture 6 μm. *a*, Free-running membrane potential (values in mV) before and after flashes (↑; first flash 25 mJ ($InsP_3$ released, 5 μM), 18 min after impalement; second flash 55 mJ ($InsP_3$ released, 10 μM), 31 min after impalement; total duration of impalement, 51 min). Voltage clamp cycles (masked from trace) were run during the barred periods (cross-referenced to *b*, *c* by symbol). Timescale, 1 min. *b*, Current trajectories recorded during the periods indicated in *a*. Current zeros indicated to left. Clamp cycle (above): conditioning voltage, −50 mV; test voltages (8), −120 to −250 mV; tailing voltage, 0 mV. Scale bar: vertical, 50 μA cm^{-2} or 300 mV; horizontal, 500 ms. No effect of $InsP_3$ on the kinetics for activation by voltage was observed. Between flashes the K^+ current varied by less than 20%. *c*, Steady-state K^+ currents (including current at −50 mV) from *b* after subtracting the instantaneous currents (see Fig. 1). Note the cumulative shift of around −100 mV in activation potential (carats, cross-referenced by symbol) recorded for the K^+ current. *d*, Steady-state K^+ currents from a second cell as in *c*, but after loading with 20 mM EGTA to buffer $InsP_3$-induced changes in cytoplasmic free Ca^{2+} concentration. Symbols: ■, control; △, following first flash (25 mJ; $InsP_3$ released, 5 μM), 17 min after impalement; ▽, following second flash (55 mJ; $InsP_3$ released, 10 μM), 32 min after impalement; total duration of impalement, 39 min. No effect of the buffer on current activation kinetics nor, before $InsP_3$ release, on membrane potential or steady-state current (not shown) was observed. Comparable results were obtained from two other cells impaled with EGTA-containing microelectrodes, and also when the Ca^{2+} buffer BAPTA[24] was substituted for EGTA.

Our findings clearly demonstrate that guard cells are competent to use $InsP_3$ in controlling K^+ channel activity. The data now also raise the possibility that $InsP_3$-mediated Ca^{2+} release forms another link in the ABA transduction chain. An immediate issue is whether the phytohormone promotes $InsP_3$ turnover. There is, further, a need to address the possible concurrent functioning of other signalling pathways in the guard cells; indeed, ABA also activates outward-rectifying K^+ channels in the guard cells[13], and neither $InsP_3$ nor cytoplasmic Ca^{2+} (ref. 9) seems to have a significant effect on these currents. ☐

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Elevation of cytoplasmic calcium by caged calcium or caged inositol trisphosphate initiates stomatal closure

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STOMATAL pores at the leaf surface regulate water loss during transpiration and CO_2 uptake for photosynthesis through changes in the turgor of the surrounding guard cells. Such regulation occurs in response to a wide range of environmental stimuli (such as light, CO_2 level and abscisic acid). These are thought to be transduced through changes in cytosolic calcium levels[1–3], and several treatments causing stomatal closure are accompanied by increases in cytosolic Ca^{2+} (ref. 4), probably from mobilization of intracellular Ca^{2+} stores. We report here the use of the fluorescent Ca^{2+} indicator Fluo-3 (refs 5, 6) to follow changes in stomatal aperture and cytoplasmic Ca^{2+} concentration as Ca^{2+} or inositol 1,4,5-

trisphosphate (InsP$_3$) are released into the cell from their caged (photoactivatable) forms[6–9]. Increasing Ca^{2+} concentration to greater than ~600 nM induced stomatal closure. Similarly, release of InsP$_3$ initiated influx of Ca^{2+} into the cytosol which was followed by stomatal closure. These results suggest that Ca^{2+} and InsP$_3$ may act as second messengers leading to guard cell closure.

Fluo-3 is a fluorescent Ca^{2+} indicator which has been successfully used to monitor changes in cytoplasmic Ca^{2+} levels ([Ca^{2+}]$_i$) in animal cells[7]. Increases in [Ca^{2+}]$_i$ lead to increases in Fluo-3 fluorescence intensity. This allows approximate calibration of [Ca^{2+}]$_i$ (refs 5, 6). The dye does not show a spectral shift on binding Ca^{2+} and so cannot be used for ratio analysis of Ca^{2+} levels[10]. Fluo-3 can, however, be used to monitor continuously [Ca^{2+}]$_i$ in cells loaded with caged probes as its excitation wavelength (490 nm) does not interfere with the cages. The caged probes are photolysed by the ultraviolet light used to excite the fluorescence of ratio Ca^{2+} probes such as Indo-1 or Fura-2 (ref. 10). Figure 1 shows typical traces of guard cells simultaneously microinjected with Fluo-3 and Nitr-5 (caged Ca^{2+}) (refs 6–8). The loaded guard cells were exposed to pulses of ultraviolet light of varying duration to photolyse different amounts of the caged Ca^{2+} and hence increase [Ca^{2+}]$_i$ to different levels. The loading of these cells with the caged probe did not lead to changes in stomatal aperture or [Ca^{2+}]$_i$ (Fig. 1*a*). When, however, [Ca^{2+}]$_i$ was elevated to greater than ~600 nM by ultraviolet photolysis of the caged Ca^{2+}, stomatal closure subsequently occurred ($n = 27$) (Fig. 1*b*, *c*). Thus a threshold of Ca^{2+} activation for the ion fluxes leading to stomatal closure is indicated. In guard cells injected with only Fluo-3, or with Fluo-3 and Nitr-5 which had been completely photolysed with ultraviolet light, application of 10- to 15-second ultraviolet pulses did not affect stomatal aperture or [Ca^{2+}]$_i$. This suggests that the observed response of stomatal closure is unlikely to be due to side effects of the photolysis procedure.

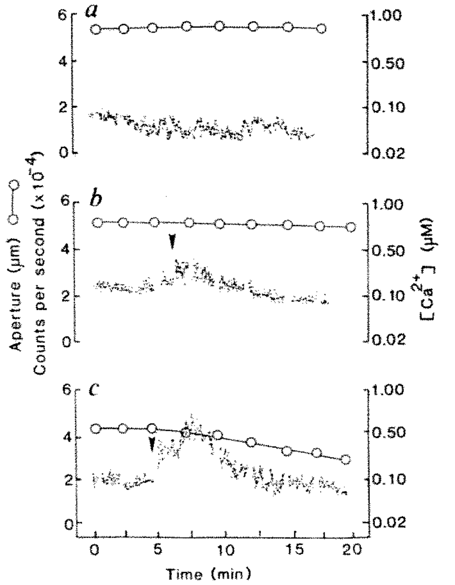

FIG. 1 Changes in [Ca^{2+}]$_i$ in guard cells of *Commelina communis* induced upon 0 s (*a*), 5 s (*b*) and 10 s (*c*) of ultraviolet photolysis (arrow) of Nitr-5 (caged Ca^{2+}).

METHODS. Abaxial (lower) epidermis of *C. communis* was peeled and fixed to a coverslip with small amounts of silicone grease around its periphery. The preparation was mounted in a microscope chamber and perfused with 50 mM KCl, 1 mM MES, 20 μM CaCl$_2$, pH 6.15, at 25 °C. Guard cells were impaled with micropipettes (tip diameters <300 nm) filled with 100 μM Fluo-3 (Molecular Probes Inc.) and 100 μM tetrasodium Nitr-5 (Calbiochem), 100 μM CaCl$_2$ and iontophoretically microinjected at less than 0.1 nA for 5–10 min. The micropipette was then removed with no obvious loss of guard cell turgor and cells incubated for 15 min to allow recovery from the microinjection procedure. Fluo-3 fluorescence (excitation 490 nm, 10 nm half bandwidth + ND2 quartz neutral density filter; emission 530 nm, half bandwidth 10 nm; dichroic mirror 505 nm; filters supplied by Ealing Electro-Optics, Watford) was measured using a Nikon Diaphot inverted microscope, Nikon CF Fluor DL 40×, 0.85 numerical aperture, 0.37 mm working distance, dry objective and a simultaneous dual emission wavelength photometry system (Newcastle Photonic Systems, Newcastle-upon-Tyne). Caged probe was photolysed using ultraviolet light from the 75 W xenon epifluorescence illuminator passed through a 350 nm (10 nm half bandwidth) interference filter (Ealing Electro-Optics). Photolysis of the caged probes did not affect guard cell autofluorescence. A 10-s ultraviolet pulse photolysed ~20% of the caged Ca^{2+} *in vitro*. Fluo-3 fluorescence was calibrated for [Ca^{2+}]$_i$ at the end of each experiment using 1 mM Mn^{2+} and 10 μM Br-A23187 (ref. 7). However, calibration of Fluo-3 fluorescence intensity against absolute [Ca^{2+}]$_i$ (refs 6,7) is extremely difficult and the values given should be taken as approximations only. The aperture of the guard cell in which the [Ca^{2+}]$_i$ was monitored was measured to 0.5 μm accuracy from micrographs taken at 2.5-min intervals throughout the experiment. Traces are representative of measurements on 10, 15 and 22 cells (Fig. 1*a*–*c*) respectively.

Ca^{2+}-activated ion efflux channels have been identified in guard cell plasma and vacuolar membranes[11–13]. These require 10^{-6} M Ca^{2+} for activation, and may represent part of the mechanism whereby elevation of [Ca^{2+}]$_i$ leads to stomatal closure. The sustained nature of the [Ca^{2+}]$_i$ increase induced by caged Ca^{2+} photolysis (Fig. 1) more closely resembles that reported for invertebrate giant axons than the quick changes seen in mammalian cells[6]. This may reflect the release of Ca^{2+} from intracellular stores induced by the elevated [Ca^{2+}]$_i$, paralleling the Ca^{2+}-induced Ca^{2+} release seen in animal cells[14].

Stimulus-evoked Ca^{2+} release from internal stores may be mediated through the release of InsP$_3$ (ref. 14) and stimulation of ^{45}Ca^{2+} efflux from plant microsomal and vacuolar prepar-

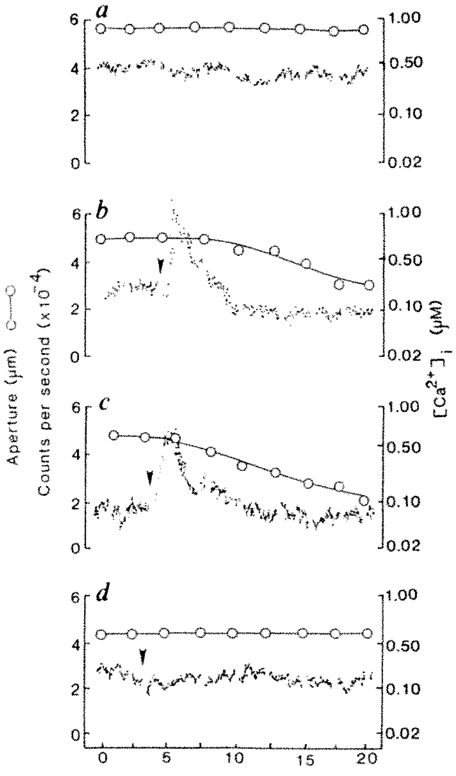

FIG. 2 Changes in [Ca^{2+}]$_i$ in guard cells of *C. communis* upon release of InsP$_3$ from its caged form. Cells were microinjected with caged InsP$_3$ and Fluo-3 as described in Fig. 1 except that the injection pipette contained 100 μM Fluo-3 and 100 μM trisodium *myo*-inositol-1,4,5-trisphosphate, $P^{4(5)}$-1-(2-nitrophenyl)ethyl ester (caged InsP$_3$) (*a*–*c*) or caged ATP (*d*). Loaded cells were subjected to 0 s (*a*) or 10 s (*b*–*d*) of ultraviolet. The cell in *b* was preincubated for 15 min in 1 mM La^{3+}. Traces are representative of measurements on 10, 18, 8 and 12 cells (*a*–*d*) respectively. Cytoplasmic Fluo-3 concentration was estimated at 10–50 μM from comparison of the Ca^{2+}-saturated Fluo-3 signal in guard cells treated with 10 μM Br-A23187 to that of 6 pl droplets (guard cell sized) of a range of [Fluo-3] in silicone oil.

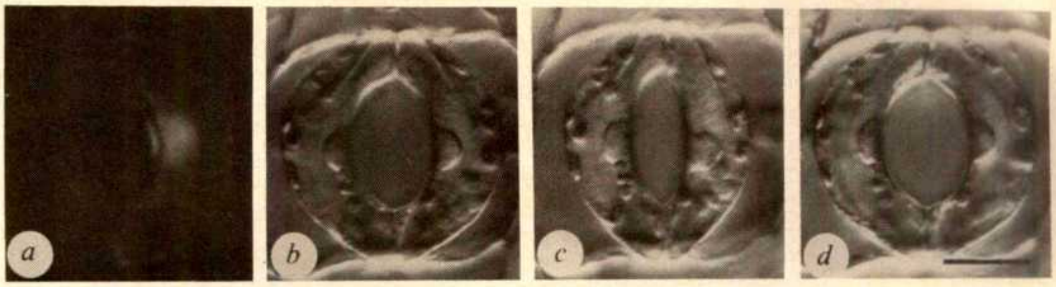

FIG. 3 Guard cell of *C. communis* microinjected with Fluo-3 and caged Ca^{2+}. *a*, Fluorescence micrograph of injected guard cell (excitation 475–505 nm, emission 520–550 nm). *b–d*, Differential interference contrast light micrographs of guard cell pair (right-hand cell only injected with Fluo-3 and caged Ca^{2+}) taken at 0 min (*b*) and 45 min (*c*) after 10 s photolysis of the caged Ca^{2+} and *d*, 120 min after the application of 10 μM fusicoccin to the guard cell in *c*. Scale bar, 10 μm.

ations by $InsP_3$ has been reported[15,16]. $InsP_3$-activated Ca^{2+} channels have also been identified in the vacuole of red beet by patch clamping[17]. We have recently found, using fluorescence ratio imaging of Indo-1, that stimuli causing stomatal closure lead to localized increases in $[Ca^{2+}]_i$, probably due to Ca^{2+} release to the cytosol from organelles (S.G., M. D. Fricker, N.D.R. and A.J.T., unpublished results). This suggested that a second messenger such as $InsP_3$ may be involved in the transduction of these closure signals. $InsP_3$ was therefore released to the guard cell cytoplasm from its caged form (inositol-1,4,5-trisphosphate-nitrophenylethyl ester, NPE-caged-$InsP_3$) and the effects on stomatal aperture and $[Ca^{2+}]_i$ dynamics investigated.

Loading cells with caged $InsP_3$ did not induce changes in stomatal aperture or $[Ca^{2+}]_i$ (Fig. 2*a*). Release of free $InsP_3$ from the cage led to stomatal closure (Fig. 2*b*) however. It also resulted in a rise in $[Ca^{2+}]_i$ over 10 s which was sustained for 5–10 min, and which preceded stomatal closure (Fig. 2*b*). Co-injecting EGTA with the caged $InsP_3$ (at 100 μM in the micropipette) abolished these effects ($n = 8$) (data not shown). An increase in $[Ca^{2+}]_i$ that preceded stomatal closure was, however, still observed when the caged $InsP_3$ was photolysed in guard cells pretreated for 15 min with 1 mM La^{3+} (Fig. 2*c*). La^{3+} inhibits Ca^{2+} influx at the guard cell plasma membrane[3], suggesting that $InsP_3$ was mobilizing Ca^{2+} from internal stores. Equivalent experiments in which NPE-caged-ATP replaced the NPE-caged-$InsP_3$ did not result in stomatal closure or increases in $[Ca^{2+}]_i$ ($n = 12$) (Fig. 2*d*), suggesting that the $InsP_3$, rather than the photolysis procedure, was responsible for the observed effects. Similarly, directly injecting inositol 1,4,5-trisphosphate into guard cells seems to lead to stomatal closure, whereas inositol 2,4,5-trisphosphate does not (not shown). Thus $InsP_3$ does seem to be acting as a messenger inducing Ca^{2+} influx into the cytosol and initiating stomatal closure. The precise intracellular site of this Ca^{2+} release is being investigated. Our previous unpublished observations using fluorescence ratio imaging of Indo-1 suggest the endoplasmic reticulum and vacuole as prime candidates. Nuclear Ca^{2+} seems extremely stable despite large changes in cytosolic levels. Thus despite its large Fluo-3 signal (Fig. 3), the variations in $[Ca^{2+}]_i$ we have observed seem less likely to have arisen in the nucleus than the cytosol.

The photolysis products of caged compounds[18] have been reported to be cytotoxic in some cell types[19] although the nitrophenylethyl ester form of caged $InsP_3$ we have used is thought to be less toxic[19]. Therefore, the viability and function of guard cells loaded with the caged probes was assessed. At the light microscope level we observed: (1) no change in organelle morphology or distribution after caged probe photolysis; (2) continuation of saltatory organelle movements in the guard cells throughout the experimental period; and (3) reversal by 10 μM fusicoccin (a fungal toxin that opens stomata[20]) of the closure induced by Ca^{2+} release from caged $InsP_3$ (Fig. 3) or caged Ca^{2+} (not shown). Also, our data from caged ATP suggests that the observed closure cannot be attributed to the cage photolysis procedure.

Thus the stomatal closure we have observed in response to photolysis of caged Ca^{2+} and caged $InsP_3$ implicates both these second messengers in the regulation of the events initiating this process. This study also presents preliminary evidence that $InsP_3$ may mobilize Ca^{2+} reserves *in vivo* in plant cells. ☐

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Bis-methionine axial ligation of haem in bacterioferritin from *Pseudomonas aeruginosa*

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THE iron-containing bacterioferritins[1–6] contain the protoporphyrin IX haem group[5]. It has been established that *Escherichia coli* cytochrome b_1, cytochrome b_{557} and bacterioferritin are identical[7]. The optical spectra at room temperature of the haem group show it to be predominantly low-spin in both the ferrous and ferric states[8]. The nature of the axial ligands binding the haem group to the polypeptide has, however, remained unknown. Low-spin, bis-coordinate haem centres in proteins typically have a role in rapid electron transfer as redox changes at the metal ion lead to little structural rearrangement[9]. There are only four amino acids with side-chains that have ligand field strengths sufficient to generate the low-spin state of haem, namely, histidine, lysine, methionine and cysteine. Hence there are, potentially, ten different pairs of these four ligands which could be discovered in electron transfer

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haemoproteins. To date only three have been established with certainty. They are bis-histidine, as in mammalian cytochrome b_5 (ref. 10), methionine-histidine, typified by cytochrome *c* (ref. 11) and lysine-histidine, recently recognized by spectroscopic methods in cytochrome *f* (ref. 12). Here we report the electron paramagnetic resonance and near infrared magnetic circular dichroism spectra of the oxidized state of *Ps. aeruginosa* bacterioferritin which enable the axial ligands to be identified as the thioether side chains of two methionine residues, a ligation scheme not previously reported for haem in any protein.

Assignment of the axial ligands of low-spin ferric protoporphyrin IX can be made, uniquely in favourable cases, using a combination of EPR and NIR-MCD spectroscopy[13]. The spectral region between 800–3,000 nm of low-spin oxidized protohaem IX contains charge-transfer (CT) transitions involving electron excitation from the highest filled porphyrin π-orbital (a_{1u} or a_{2u}) to the hole in the Fe(III) 3d-shell, which is a combination of d_{xz} and d_{yz} orbitals[14]. As the energies of the d-orbitals are controlled by the ligand field strength of the haem axial ligands the wavelength of the porphyrin-to-ferric ion CT band varies as the ligand is changed. Hence the peak wavelength of the CT band can be used to identify haem axial ligands. The CT bands are most readily measured in proteins using magnetic circular dichroism (MCD) spectroscopy[15] because the absorption spectrum in the region ~1,400–3,000 nm is partially obscured by the overtones arising from O–H, N–H and C–H vibrations. However, the intensity of the MCD signal depends, *inter alia*, upon the ground state magnetic properties and hence is several orders of magnitude weaker in the case of vibrational than electronic transitions[13]. The MCD spectrum therefore provides a method of locating low-energy electronic states without the possibility of confusion from vibrational bands. A wide range of haem proteins and model Fe porphyrins containing a variety of axial ligation states has been studied to determine the peak wavelengths of the CT bands characteristic of the ligation type and to enable unambiguous assignments to be achieved[13].

The CT band shifts in a systematic way with ligand field such that an approximate additivity rule applies[13]. For example, on changing axial ligation in leghaemoglobin *a* (soybean) from histidine-imidazole to histidine-imidazolate the CT band moves from 1,600 nm to 1,360 nm, a shift of 1,100 cm^{-1} (ref. 16), and similarly a change from methionine-histidine to methionine-histidinate coordination in *Escherichia coli* cytochrome b_{562} causes the CT band to move from 1,860 nm to 1,550 nm, an energy shift of 1,080 cm^{-1} (ref. 17). We have used this additivity rule to assign the near infrared (NIR)-MCD spectrum of *Ps. aeruginosa* bacterioferritin.

The NIR-MCD spectrum of this protein is shown in Fig. 1. The elctron paramagnetic resonance (EPR) spectrum is given as an inset. The MCD spectrum is temperature dependent, as expected from the paramagnetic state of the haem group. The form of the spectrum is typically that of a porphyrin-to-ferric CT transition[13]. The wavelength of the prominent peak at 2,230 nm is unique, however, being the longest wavelength CT band yet identified in any low-spin ferrihaem. No structurally characterized protein is known with a CT band at this wavelength.

TABLE 1 Electronic *g*-values and peak wavelengths of CT bands of bacterioferritins and bis-thioether Fe(III) porphyrin complexes

	g-values	Wavelength maximum of CT band in MCD
Bacterioferritin		
Ps. aeruginosa	2.86, 2.32, 1.40	2,240 nm
A. vinelandii	2.88, 2.31, 1.46	2,270 nm
E. coli	2.88, 2.31, 1.46	2,270 nm*
R. sphaeroides	2.87, 2.32, 1.48	ND
Model compounds		
I	2.90, 2.37, 1.47	ND†
II	2.90, 2.29, 1.43	2,130 nm‡

Compound I, bis(tetrahydrothiophene) (meso-tetraphenylporphinato)-iron(III)-carborane; II, bis(tetrahydrothiophene) (octa-ethylporphinato)-iron(III)-perchlorate. ND, not determined.

* M.R.C., S. C. Andrews, P. M. Harrison, J. R. Guest, G.R.M., A.J.T., F.K. and C.G., unpublished data.

† Ref. 18.

‡ J. McKnight, C. A. Reed, M. R. C and A. J. T., unpublished data.

We deduce that bis-methionine coordinated protohaem IX will have a CT band in the observed wavelength region. Bis-histidine ligation gives rise to a CT band between 1,500 and 1,630 nm, depending on the protein environment, and methionine-histidine coordination leads to a CT band between 1,750–1,950 nm. Taking the mean wavelength positions of the CT bands this is a shift of 990 cm^{-1} to the red on replacing one histidine by one methionine group. Exchange of a second methionine ligand should cause a further shift to the red of ~1,000 cm^{-1}, yielding a predicated energy of the CT band of bis-methionine coordinated haem of 4,400 cm^{-1}, which is a mean wavelength of 2,270 nm. The range of wavelengths predicted are shown in Fig. 1. It can be seen that the measured wavelength of the peak falls well within the expected range. No other ligation scheme predicts the placement of the corresponding band in this region. This ligation is confirmed by the NIR-MCD spectrum of the model bis-thioether complex, that is, bis-tetrahydrothiophene-(octaethylporphinato) iron (III) perchlorate, which shows a CT band at 2,130 nm. (Table 1).

The EPR spectrum lends further support to this assignment. The inset in the figure shows the spectrum of *Ps. aeruginosa* bacterioferritin. The peaks indicated by *g*-values can be assigned to the low-spin ferric haem group. The table compares the *g*-values determined for bacterioferritin from four different bacterial sources and the *g*-values of two model bis-thioether Fe(III)

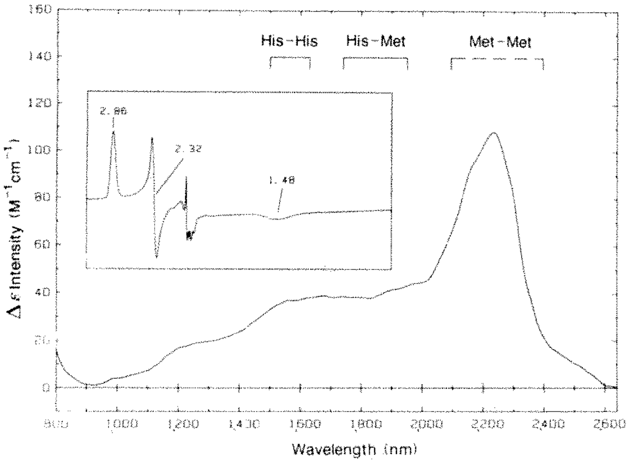

FIG. 1 The MCD spectrum of *Ps. aeruginosa* bacterioferritin at 5 Tesla and 4.2 K. $\Delta\varepsilon = \varepsilon_L - \varepsilon_R$, where ε_L and ε_R are the molar extinction coefficients for left and right circularly polarized light, is estimated per haem group determined by double integration of the EPR spectrum. The method of measurement is given in ref. 13. The inset shows the EPR spectrum of the same sample in the region of the low-spin ferric haem signals measured between 200 and 600 mT, at 4 K, 2 mW power, and a frequency of 9.38 GHz. Haem concentration 280 μM, 200 μM HEPES buffer (pH 7.4) in D_2O with 50% (v/v) deuterated glycerol added.

porphyrin complexes. Model compound I has been shown by X-ray crystallography to have two thioether ligands coordinated to Fe(III)[18]. The similarity between the g-values of these model compounds and bacterioferritin is striking.

The evidence from the NIR-MCD spectrum and the comparison between the g-values of the protein and bis-thioether model compounds leaves little doubt that the haem group in *Ps. aeruginosa* bacterioferritin is ligated by the thioether side chains of two methionine residues. Table 1 indicates that this seems to be a common feature amongst bacterioferritins from a variety of sources. In no bacterioferritin has a haem content of greater than 0.5 haem per protein subunit yet been observed. For example, neither *Azotobacter vinelandii*[4] nor *E.coli.*[20] bacterioferritin apparently contains more than 0.5 haem per subunit and the protein from *Ps.aeruginosa* is reported to have only one haem per five subunits[5]. If 0.5 haem per subunit is the maximum number of haem groups which can bind to bacterioferritin, either the haem group is bound at the interface between a pair of subunits or only half the subunits bind haem. The identification of the haem ligands as methionine does not, at this stage, rule out the latter possibility, but we consider it most likely that the haem group is bound by individual subunits, as is found in other multimeric haemoproteins.

Andrews *et al.*[19] have reported the amino-acid sequence of *E. coli* bacterioferritin and a secondary structure analysis which indicates a high α-helical content consistent with a structure composed of bundles of four α-helices. Such a tertiary structure for bacterioferritin is consistent with its similarity to mammalian ferritins[1], shown by X-ray crystallography to consist of bundles of four α-helices[1,21]. *E. coli* cytochrome b_{562} (ref. 22) and *Rhodospirillum molischianum* cytochrome c' (ref. 23) also have a four-α-helix bundle structure. In these two proteins the haem lies between two pairs of helices so that it is located at one end of the bundle with the haem normal perpendicular to the bundle axis. On the basis of the analysis of Andrews *et al.*[19], a similar location for the haem of bacterioferritin is possible, involving ligation in the *E. coli* protein by Met 1/Met 144 near the N and C termini of the polypeptide chain. Ligation by Met 31/Met 119 or Met 120 may be sterically possible. A partial sequence of the related protein from *Nitrobacter winogradskyi*[24] shows that Met 31 is not conserved, however, and hence cannot be a ligand, suggesting that the Met 1/Met 144 site is likely. ☐

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Implications of thermodynamics of protein folding for evolution of primary sequences

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NATURAL proteins exhibit essentially two-state thermodynamics, with one stable fold that dominates thermodynamically over a vast number of possible folds, a number that increases exponentially with the size of the protein. Here we address the question of whether this feature of proteins is a rare property selected by evolution or whether it is in fact true of a significant proportion of all possible protein sequences. Using statistical procedures developed to study spin glasses, we show that, given certain assumptions, the probability that a randomly synthesized protein chain will have a dominant fold (which is the global minimum of free energy) is a function of temperature, and that below a critical temperature the probability rapidly increases as the temperature decreases. Our results suggest that a significant proportion of all possible protein sequences could have a thermodynamically dominant fold.

We investigate a model of a protein chain in which protein folds are characterized by sets of coordinates of C_α atoms $\{r_i^m\}$ where index i denotes the position of an amino-acid residue in the sequence and m denotes the fold. Monomers are positioned in sites of a three-dimensional lattice to account for steric interactions. Energy of a fold m, say, is represented in a simple form:

$$E_m = \sum_{i,j}^{N} B_{ij} \Delta(r_i^m - r_j^m) \qquad (1)$$

where N is the total number of monomeric units, B_{ij} is the interaction energy between monomers i and j when they are adjacent in space; this energy depends on the types of these monomers. $\Delta(r_i^m - r_j^m) = 1$ if monomers i and j are lattice neighbours and 0 otherwise.

The Boltzmann probability of a fold p_m is given by

$$p_m = \exp(-E_m/k_B T)/Z$$

where $Z = \sum_m^M \exp(-E_m/k_B T)$ is the partition function of the chain. M is the total number of folds; it grows exponentially with N so that $M = \gamma^N$ and γ is the number of conformations per monomer.

The existence of a unique structure of a protein implies that one fold m_0 corresponding to this structure dominates thermodynamically over all other folds so that:

$$p_{m_0} = 1 - \varepsilon \qquad (2)$$

where $\varepsilon \ll 1$.

To determine the probability that a random protein folds into a unique structure, we assume the interaction energies B_{ij} to have random values. There are 20 types of amino-acid residues and, hence, $210 \gg 1$ types of interactions between them. Therefore we assume the probability distribution of B_{ij} to be

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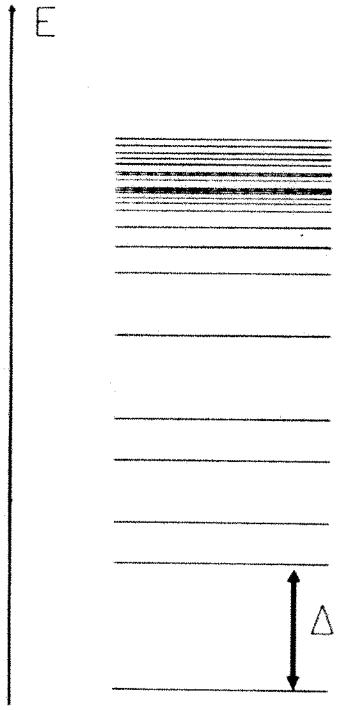

FIG. 1 Typical energy spectrum of a protein. Each line corresponds to a fold. The total number of energy levels is the same as the number of folds, γ^N. The energy of each fold has a random value as we consider a random realization of a sequence. The vast majority of folds belong to the top quasicontinuous part of the spectrum, which corresponds to disordered conformations with energies lying in the range $(\bar{E} - B\sqrt{2\rho N}, \bar{E} + B\sqrt{2\rho N})$. Few levels ($\sim N$) lie in the bottom part of the spectrum; these levels correspond to folds with best-fit contacts and have energies $\sim \bar{E} - Ne$ where $e = B\sqrt{2\rho \ln \gamma}$. At high temperature the energy of a chain corresponds to the top part of the spectrum which is entropically favourable; decrease of temperature leads to the movement downwards of the spectrum and at some definite temperature, T_c, a transition takes place to the bottom discrete part of the spectrum which is entropically unfavourable but favourable energetically. The density of levels in the discrete part of the spectrum is so low that the protein cannot jump from level to level (from fold to fold) by thermal fluctuations. This is the reason for the freezing transition at $T = T_c$ when only a few lowest-lying states become available to the protein. The transition temperature T_c is universal for all sequences but the actual number of thermodynamically relevant folds at $T < T_c$ depends on specific features of the bottom part of the spectrum for a given sequence and especially on the energy gap Δ between the ground fold and the first 'excited' fold.

continuous and gaussian; that is:

$$P(B_{ij}) = \frac{1}{\sqrt{2\pi B^2}} \exp\left[-\frac{(B_{ij} - B_0)^2}{2B^2} \right] \tag{3}$$

where B_0 is the mean which determines overall compactness and B is the variance which corresponds to the heterogeneity of the chain.

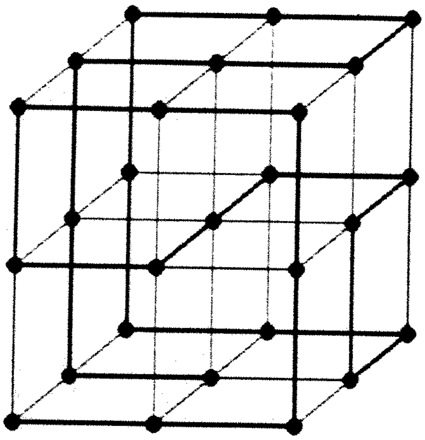

FIG. 2 The lattice and example of a compact self-avoiding walk of a chain of 27 monomers (thick line). Exhaustive enumeration by a first-depth algorithm yields all possible folds unrelated by symmetry. The total number of folds is 103,346. Two hundred realizations of sequences were generated with a distribution of contact energies between monomers B_{ij} given by equation (3). The mean B_0 is unimportant as we consider compact conformations with fixed number of contacts and this parameter gives a constant contribution to energy of each conformation. Therefore we assumed $B_0 = 0$. Standard variance B determines T_c. We estimated it according to data on inter-residue contact energies in proteins given in ref. 8: $B = 0.6\,\text{kcal m}^{-1}$ which yields $k_B T_c \approx 0.9\,\text{kcal m}^{-1}$. But the particular value of T_c is also not crucial here since we use dimensionless parameter T/T_c. Energy of each fold for each sequence was calculated according to equation (1) in which only nearest neighbours in space were assumed to interact. Boltzmann probabilities p_m ($m = 1, \ldots 103{,}346$) of all folds for 200 realizations of sequences (sets of B_{ij}) were determined and parameter X (equation (5)) was evaluated for each sequence at different temperatures.

The thermodynamics of the chain is completely determined by the set of energies of all folds E_m, that is, by the energy spectrum (see Fig. 1). The statistical properties of this energy spectrum were obtained in ref. 1 where the microscopic model defined by equations (1) and (3) (with polymeric bonds taken into account explicitely) was investigated analytically by a replica approach. The result reads that energies of different folds can be treated as statistically independent random values with a gaussian distribution:

$$P(E_m) = \frac{1}{\sqrt{2\pi\rho N B^2}} \exp\left(-\frac{(E_m - \bar{E})^2}{2\rho N B^2} \right) \tag{4}$$

(ρ is the average number of contacts between residues) and different low-energy folds (bottom lines of the spectrum) of the same sequence are structurally different (this is the reason why their energies are independent random values).

These results demonstrate the equivalence between disordered heteropolymers and the random energy model (REM) introduced by Derrida[2] in the theory of spin glasses. (This equivalence had been postulated *a priori* in ref. 3).

The parameter which gives the effective number of thermo-

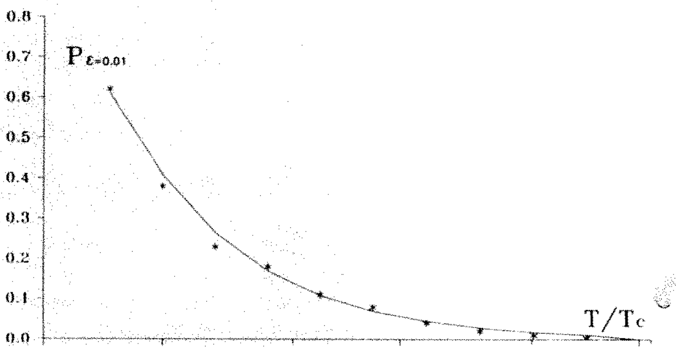

FIG. 3 Fraction of sequences that are able to fold into unique structure, that is, having Boltzmann probability of ground state $p_{m_0} > 0.99$ (or $X < 0.02$) plotted against T/T_c. The solid line denotes the analytical result of equation (6), asterisks denote the numerical results obtained from exhaustive enumeration of all conformations for 200 sequences.

dynamically relevant folds is

$$X = 1 - \sum_{m=1}^{M} p_m^2 \tag{5}$$

(note that $\sum_{m=1}^{M} p_m = 1$). When one state m_0 dominates, $p_{m_0} \approx 1$ and $X \approx 0$. In the opposite case when all microstates have equal Boltzmann probabilities, $p_m = \gamma^{-N}$ and $X = 1 - \gamma^{-N} \approx 1$.

The equivalence of random heteropolymers to the REM gives the main clue to the estimation of the probability that a random chain will fold. The probability distribution of X had been calculated for spin glasses[4] and explicitly for the REM[5]. (For the general case of a disordered system that possesses an energy spectrum as shown in Fig. 1, with quasicontinuous top part and discrete bottom part, the same probability distribution has been derived[6].)

Using these results, it is easy to derive the probability P_ε that for a random chain a ground fold m_0 will dominate, that is, $p_{m_0} > 1 - \varepsilon$ and, hence, to the same order in ε, $X < 2\varepsilon$:

$$P_\varepsilon = \frac{\sin(\pi X_0)}{\pi X_0} \varepsilon^{X_0} \tag{6}$$

where X_0 is X averaged over all sequences[4,5].

X_0 was found[1] as a function of temperature, T:

$$X_0 = \begin{cases} T/T_c & \text{if } T/T_c < 1 \\ 1 & \text{if } T/T_c \geq 1 \end{cases} \tag{7}$$

where

$$T_c = \frac{B\sqrt{\rho}}{2k_B\sqrt{\ln \gamma}} \tag{8}$$

T_c is the temperature when the chain backbone obtains a unique conformation. It may not coincide with T_d, the temperature of denaturational transition when fixation of rotational isomers of side chains and their tight packing occurs[7]. When $T_c > T_d$, a 'molten globule' with a unique backbone conformation would exist in the temperature range $T_c > T > T_d$.

Equations (6)–(8) give the main result of this work: at high temperatures, $T > T_c$, $X_0 \equiv 1$ therefore (see equation (6)) $P_\varepsilon \equiv 0$ and there are no sequences which can fold. When the temperature decreases below T_c the fraction of sequences that are able to fold grows drastically. For example, taking $\varepsilon = 0.01$, which corresponds to 99% ground fold dominance, we obtain $P_\varepsilon \approx 0.1$ at $T = T_c/2$. This means that under this condition every tenth sequence will have one fold with 99% dominance.

This result is universal: all microscopic characteristics of folded chains such as heterogeneity B, entropy per bond $\ln \gamma$, coordination of monomers ρ, are introduced via the single parameter T_c so that the main result, equation (6) depends only on the dimensionless parameter $X_0(T/T_c)$. It should be emphasized also that the 'folding capacity' of a random chain does not depend on its length, N. The above investigation implies also that folding of a random chain could lead to the global minimum of free energy.

We tested the analytical result equation (6) by exhaustive enumeration of all self-avoiding compact conformations of a 27-monomer chain in a 3*3*3 fragment of a simple cubic lattice (Fig. 2). The results (Fig. 3) match the analytical expression in equation (6).

We conclude that the requirement for folding may not be very restrictive for evolutionary choice of protein sequences. □

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Biolistic transformation: microbes to mice

S.A. Johnston

A new design of the Biolistic device allows the *in-situ* transformation of animal tissue and improved efficiencies of transformation of plants and microbes.

For all the advances in recombinant DNA technology, the introduction of genetic material into the species or cells of interest often remains a rate-limiting step. In this regard, the Biolistic — biological and ballistic — process (Du Pont, Wilmington, Delaware) has proved to be a valuable new development. The device and procedure were originally applied to plants, and a wide variety of plant species have now been transformed[1], both transiently and stably. Recently, it was announced that the Biolistic process had been used to accomplish the long-awaited stable transformation of corn. The process has also been effective in transforming microbes, both eukaryotic[2] and, more recently, prokaryotic (J. Sanford, personal communication). Another first for the procedure was the stable transformation of mitochondria[3]. Biolistic transformation of microbial[4] and plant[5] chloroplasts has been reported.

Principles of operation

The original publication on biolistic transformation described several possible designs[6]. The instrument (PDS-1000) used in early experiments is now manufactured by Du Pont. The procedure involves first coating the DNA on microbeads composed of tungsten or gold. The size of the beads used ranges from 0.5 to 5 μm depending on the dimensions and penetrability of the target cells. A few microlitres of a slurry of these microprojectiles are placed on the surface of a macroprojectile, which is accelerated down a barrel by a gun-powder discharge. A stopping plate at the end of the barrel stops the macroprojectile, but a small hole in the plate allows the microprojectiles to proceed towards the target cells. The bombardment takes place in a vacuum chamber in order to lessen the impediment of air on the velocity of the microprojectiles. Several variations on this theme have been developed as instruments[7-9] and at least one of these has been used for important advances in plant transformation[10]. Each of these designs involves placing the target in a partially evacuated chamber.

Animal cell transformation

An obvious question is whether the Biolistic technology can be extended to animal cells. Zelenin *et al*[11] was first to report the use of microprojectile bombardment to transform cultured mouse NIH 3T3 cells. Several other types of cultured cells have been transformed including human HT1080 and 143B (J. Sanford, personal communication), CHO, EL4 (S. McElligott, personal, communication) and chick fibroblasts (D. Armaleo, personal communication). We have transformed primary cultures of both skeletal myotubes and cardiac myocytes, as well as pigmented epithelial cells. The transformation of the skeletal myotubes was highly efficient, often with greater than 10 per cent of the cells expressing the transgene product. The biolistic transformation of myotubes was 20–100 times as efficient as transformation by $CaPO_4$ or lipofection. Interestingly, the expression was stable for the culture life of the cells (~7 days), even though they were mitotically inactive (R.S. Williams, submitted).

Bombardment of cultured cells in the PDS-1000 leaves the centre portion of the culture heavily damaged by ballistic trauma. The helium-driven device discussed below alleviates this problem, leaving greater than 95 per cent of the cells viable. This modification has also led to significant improvements in the total expression per plate (~15-fold). These results indicate that the Biolistic transformation protocol may be useful in transforming cultured animal cells, particularly primary cells or cells recalcitrant to transformation by conventional techniques.

Helium-driven 'wand' design

One of the applications of the Biolistic technique that was anticipated was the direct transformation of tissues and organs in intact living animals[12]. This goal necessitated reconfiguration of the device so that (1) it could be hand-held, (2) it would impart a high velocity to the microprojectiles, and (3) it would be non-traumatic to the tissue. The basic features of the apparatus fulfilling these demands is shown in Figure 1. The impelling force is from a shock-wave generated by bursting a membrane holding back high-pressure helium. The macroprojectile is a thin (2 mil) Kapton disc on which the DNA-coated microprojectiles have been spread. The macroprojectile is stopped by a screen in such a fashion that the target tissue is largely sealed-off from the expanding gas. The stopping screen is close to the target surface so that it is not necessary to create a vacuum in the flight-path of the microprojectiles, although it is possible to do so.

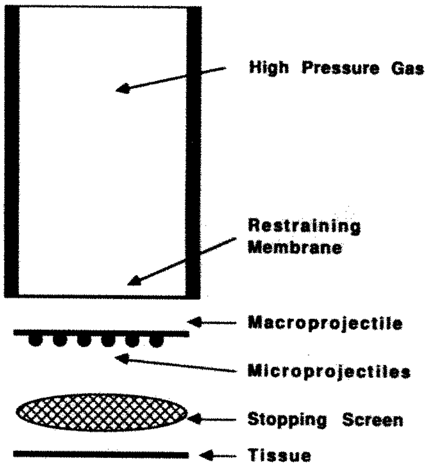

FIG. 1 Principles of operation of the helium-driven wand.

Using this 'wand-like' device we have successfully transformed skin, liver, spleen and intestine in living mice. The expression has been transient, using luciferase or human growth hormone as a reporter. Transgene activity has extended for up to 14 days in skin and 23 days in liver. Histological examination of the bombarded area reveals that there is relatively little damage to the tissue and that the DNA is carried into the tissue with the microprojectiles. As many as 25 per cent of the cells in the bombarded area of skin will express the transgene product (S.A.J., submitted).

The wand-like device has been fitted onto the standard PDS-1000 (see Fig. 2) producing surprising results. For every target tested the helium-driven adaption gave a 5–100-fold improvement in transformation efficiency. Yeast, bacteria, plant and mammalian cells have all responded well to the new adaption.

Future prospects

The ability to transform animal tissue *in situ* offers many opportunities. Even transient expression of transgenes could be very useful. For example, delineation of tissue-specific *cis*-acting elements could be performed directly in the authentic setting, rather than relying on transient expression assays in cell lines. Some somatic cell therapies may require only

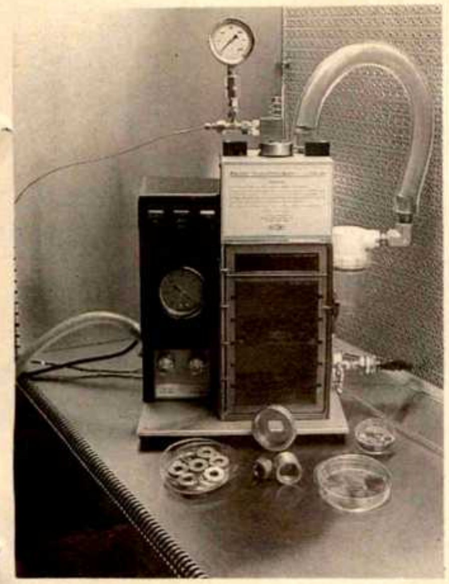

FIG. 2 Prototype of the PDS-2000 helium-driven device.

short-term expression of some factor — for example, tissue plasminogen activator after a heart attack. Transient expression may elicit an antigenic response, making a form of genetic immunization possible.

Most somatic cell therapies, however, envision long-term or permanent expression of the transgene. The usefulness of the Biolistic technique for these applications will depend on the ability to target enough stem cells and the efficiency of integration of the introduced DNA. Whether the Biolistic device and the vectors will be adequate for these requirements remains to be seen. However, given the major contributions this technology has made to plant and microbial systems in the three years since its introduction, it seems likely that the same will be true for animal applications. ☐

Stephen A. Johnston is an Associate Professor at 139 Biological Sciences, Duke University, Durham, North Carolina 27706, USA. Du Pont is developing a helium-driven modification of the PDS-1000 but has at this time no specific timetable for the production of the wand-like device used in our in vivo *transformation experiments. For more information, fill in reader service number 100.*

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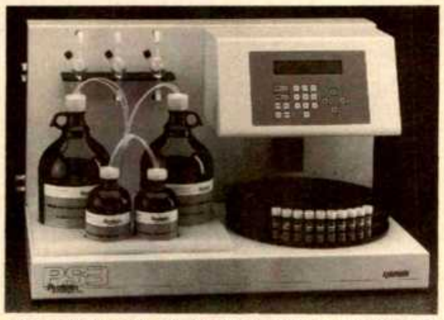
Washington's chemistry set

Featured at next week's American Chemical Society meeting to be held in Washington, D.C. will be a structure prediction program for NMR spectroscopists and chromatography software for IBM PCs or compatibles.

ACCORDING to Rainin Instruments, reduced solvent consumption, fast coupling and easy operation are features of the Model PS3 **automated peptide synthesizer** (*Reader Service No. 101*). Designed for unattended overnight operation if required, the PS3 can synthesize up to three separate peptides at a time, with cycle times of only 32 minutes per amino acid, says Rainin. The instrument, which sells for under $30,000 (US), uses FMOC chemistry and is designed to produce high yields without the use of hydrogen fluoride. Amino acids can be activated directly in the reagent vials, which eliminates the complex plumbing associated with conventional instruments. Reliability is assured with the PS3's inert zero dead-volume valving system. For more information on Rainin's product range, see booth 1014.



Rainin's Model PS3 will synthesize peptides while you sleep.

Retrovirologists may be interested in a new **simian immunodeficiency virus (SIV) p27 core antigen assay** from Coulter for the determination of SIV in plasma, serum and culture media (*Reader Service No. 102*). The assay uses a monoclonal capture EIA, similar to Coulter's HIV p24 antigen assay. Antigen that is 'captured' by anti-SIV p27 antibody after an overnight incubation is measured using the biotin/streptavidin/peroxidase detection system. The assay can be used to provide qualitative results by using positive and negative controls. Alternatively, the relative amount of antigen can be determined from a standard curve.

A collection of 67 papers on various topics in the field of **lectinology**, which were presented at the *10th International Lectin Meeting* held in Prague, Czechoslovakia, 1988, are now available in one volume from Sigma entitled *Lectins: Biology, Biochemistry, Clinical Biochemistry*, Vol. 7 (*Reader Service No. 103*). This 462-page hard-back book includes articles by over 180 scientists in this specialized area of research.

Instrumental ideas

Spex Industries offers the CD6 **circular dichroism spectropolarimeter system**, which automates the characterization of a compound's optical activity (*Reader Service No. 104*). As the CD6 is designed to operate with high performance down to 180 nm, it is useful for conformational studies such as the secondary structure analysis of proteins, polypeptides and polynucleotides. The instrument, which sells for under $70,000 (US), has menu-driven software and automated control of all instrument parameters. It can be used with cylindrical, rectangular or micro-cells, without the need for adaptors. Spex also supplies a range of optional extras. Visit Spex in booth 1009.

The one-step **environmental extractor/concentrator apparatus** from Corning is designed to save time and cut costs (*Reader Service No. 105*). Made of Pyrex, the integrated system does not require sample transfer between extraction and concentration and can easily be dismantled for cleaning. The apparatus comes with inbuilt solvent recovery. Corning will be showing off its wares in booth 549.

The IR-Plan family of **FT-IR microscopes** from Spectra-Tech allow chemists and other scientists to choose the level of sampling flexibility most suited to laboratory requirements (*Reader Service No. 106*). Three models are available: IR-Plan Research, Analytical and Laboratory microscopes. Patented resolution enhancement features — redundant aperturing, targeting and sample compen-

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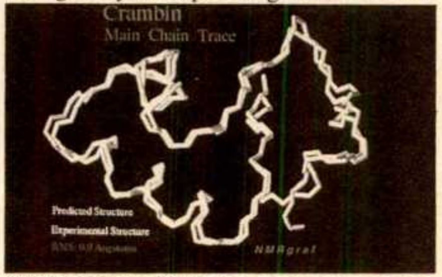
Reader Service No.25

sation — ensure, says Spectra-Tech, that the sample seen and apertured is the exact sample that is represented in the infrared spectrum. Prices range from about $19,500 to 38,000 (US). Stop by the Spectra-Tech booth, number 1220.

Computing corner

NMRgraf — the latest software tool for NMR spectroscopists available from BioDesign — is a **structure prediction program**, which uses data from NMR experiments in conjunction with computer-aided molecular design techniques, to produce and display molecular structures in 3-D (*Reader Service No. 107*). NMRgraf operates on multiple hardware platforms and typical application areas include protein and polymer research. When used with Nuclear Overhauser Effect (NOE) and J-Coupling data, NMRgraf provides a fast and accurate way of predicting low-energy structures consistent with experimental data, says BioDesign. By incorporating data from the NMR experiment with molecular simulation, NMRgraf can be used to model most molecules, even though the structure or a related structure may not be in a database such as Brookhaven or Cambridge. NMRgraf sells for between $40,000 and 150,000 (US) depending on CPU performance, graphics options and software modules, and can be seen in booth 814.



NMRgraf from BioDesign can compare predicted structures with experimental data.

Version 2.22 of Lab Calc — **software for chromatographers** — is now available from Galactic Industries (*Reader Service No. 108*). Lab Calc runs on IBM PCs, PS/2s and compatibles and is a useful tool for both novice and advanced users alike. The upgraded version has a method editor, which simplifies the development of advanced chromatography methods. Lab Calc has a baseline correction feature that can subtract complex polynomial baselines from data. In addition, raw data can be adjusted for differences in run-to-run retention times and various smoothing, derivative smoothing and interpolation functions can be used to examine chromatograms. Chromatogram peaks can be zoomed in real time, peak positions, peak edges, amplitudes, areas, percent areas, concentrations or compound names can be displayed, and chromatograms can be overlaid to facilitate comparison. The software supports up to eight independent channels for data acquisition. Data channels can be viewed together or independently or, alternatively, data collection can proceed in the background leaving the PC free to process data. See the $1,490 (US) Lab Calc package in booth 1203.

Chemical Design's first public demonstration of its Chem-X **molecular modelling software** running on Silicon Graphics 4D Iris workstations can be seen at the ACS meeting (*Reader Service No. 109*). Workstations such as these, which are widely used by protein and polymer chemists, form a complement to the networked VaxStation and Apple computer solutions already offered by Chemical Design. Beta-testing of the product will begin in October and full release is expected by the end of the year. The annual cost of an entry-level, single-user Chem-X system for a Silicon Graphics workstation will be $8,000 (US). See Chem-X in booth 507.

For the real-time viewing and manipulation of chromatograms and hard disk data storage, Perkin-Elmer Nelson recommends its Model 1020 **personal integrator** (*Reader Service No. 110*). Occupying just 12 inches of benchtop space, the Model 1020 with its built-in screen can be interfaced to all commercial liquid and gas chromatographs and is compatible with PCs and larger systems. Using the windows-based software, users can view one or two real-time plots, change attenuation, and examine stored or sample chromatograms in detail or compared against reference standards. Chromatograms can be stored on the integrator's hard disk or saved on microdiskettes for more permanent archival storage. Perkin-Elmer will be in booth 615 at the ACS meeting.

Model 1020 LC/GC personal integrator.

These notes are compiled by Diane Gershon from information provided by the manufacturers. To obtain further details, use the reader service card bound inside the journal. Prices quoted are sometimes nominal, and apply only within the country indicated.

THE DEVELOPMENT OF THE LABORATORY

ESSAYS ON THE PLACE OF EXPERIMENTS IN INDUSTRIAL CIVILIZATION

Edited by Frank A J L James
Secretary & Research Fellow, Royal Institution Centre for
the History of Science and Technology, London

Laboratories are now fundamental to the practice of science, yet there is a paucity of serious historical analysis of the subject. There are, and have been, a wide variety of laboratories, and the multiplicity of their development allows many different approaches to analysis. The contributions in this book reflect this diversity and will allow all who are interested in laboratories to better understand their place in the advancement of science.

Contents – Introduction – *F A J L James (Royal Institution, London)* / **CHEMICAL LABORATORIES** / Humphry Davy: Fund Raiser – *J Z Fullmer (Columbus, Ohio)* / The Lectureship in Chemistry and the Chemical Laboratory, University of Glasgow, 1747-1818 – *D V Fenby (Dunedin, New Zealand)* / Amusement Chests and Portable Laboratories – *B Gee (Saltash, Cornwall)* / **THE EXTENSION OF LABORATORIES TO PHYSICS** / Techniques from Texts: Recovering the Micro-structure of Real Experiments – *D Gooding (University of Bath)* / A Physics Laboratory at Harvard University – *L Aronovitch (MIT, Cambridge, Mass.)* / J J Thomson and 'Cavendish' Physics – *I Falconer (Royal Institution, London)* / The Astronomical Observatory as Practical Space: The Examples of Bessel and Struve – *M E W Williams (LSE, London)* / The Geopolitics and Architectural Design of a Metrological Laboratory: The Physikalisch-Technische Reichsanstalt in Imperial Germany – *D Cahan (Nebraska)* / The Laboratories of Finsbury Technical College in the 1880s – *W H Brock (University of Leicester)* / **THE LARGE PHYSICS LABORATORIES** / Pragmatism in Particle Physics: Scientific and Military Interests in the Postwar United States – *A Pickering (Illinois)* / Fermilab: Founding a $250 million Accelerator – *C Westfall (Michigan)* / The CERN Beam Transport Programme in the Early Sixties – *J Krige (CERN, Geneva)* / 'Monsters' and Colliders in 1961: The First Debate at CERN on Future Accelerators – *D Pestre (CERN, Paris)* / Index

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Applicants should hold a doctoral degree and have a strong record of original research. Preference will be given to applicants at the level of Assistant Professor, but candidates at more senior ranks will also be considered. Successful candidates will join an interactive and diverse faculty and participate in vital campus-wide graduate programs.

Send curriculum vitae, a statement of research background and interests, and three letters of recommendation to: **Dana Carroll, Faculty Search Committee, Department of Biochemistry, University of Utah School of Medicine, Salt Lake City, UT 84132.**

Deadline for applications is December 1st, 1990; late applications will be considered if openings are still available. Equal opportunity, affirmative action employer.

(NW5653)A

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Department of Biochemistry
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This position is available from 1st October, 1990 within the research group of Professor R M Denton and is funded by long-term support from the Medical Research Council until at least 30th September, 1993. The research of this group is concerned with establishing the mechanism of action of insulin. The successful applicant would be expected to study the role of activated serine protein kinases including acetyl-CoA carboxylase kinase. The salary will be on the grade IA scale according to age and experience. Experience with the study of protein phosphorylation would be an advantage but not essential.

Informal enquiries should be made to Professor R M Denton (0272 303523 direct line).

For further details telephone Bristol 303136 (ansaphone after 5.00pm) or write to the Personnel Office, Senate House, Bristol BS8 1TH. Please quote reference 306. Closing date for applicants 17th September, 1990.

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(4430)A

UNIVERSITY OF UTRECHT
The Netherlands

Faculty of Biology, Department of Plant Ecology and Evolutionary Biology

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Professor of Landscape Ecology (m/f)

The Department executes research and provides education in a broad spectre of botanical, ecological and evolutionary subjects. The section Landscape Ecology comprises of 4 tenured and 2 temporary positions of scientists. The section has a vigorous research programme directed at biogeochemical interactions between the ecosystems in a landscape, with special attention for nutrient transport and nutrient cycles. In this research programm the section closely cooperates with the other sections in the Department.

The candidate should be able to give guidance to this programme and stimulate its further development. The candidate also should be willing on teach biology students at the pre-graduate, graduate and postgraduate levels. Teaching should be carried out in the Dutch language within two years after accepting the position. The candidate should be willing to participate in management and administrative tasks.
The candidate should have completed a Ph.D. in Biology of Agricultural Sciences and an adequate publication record on relevant subjects.

Salary is in the range of 7171.– to 10.544.– Dutch guilders per month.

Information is available from the Dean of the Faculty, prof. dr M.J.A. Werger, telephone (0)30-532276.
A detailed task description (in Dutch) is available.

Applications complete with curriculum vitae and list of publications should be sent, within a month after publications of this advertisement, to the Selection Committee, Bureau of the Faculty of Biology, Padualaan 14, NL 3584 CH Utrecht, The Netherlands.

(W7671)A

With equally qualified applicants preference will be given to a woman.

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The Ohio State University
Columbus, Ohio

The Ohio State University invites nominations and applications for the position of Vice President for Research. The Vice President will report directly to the newly appointed President and work closely with the Vice President for Academic Affairs/Provost and the Dean of the Graduate School to stimulate and facilitate research and scholarship at the University. This individual will play a key role in the growth of research activities at the University.

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To assure full consideration, applications and nominations should be received by October 8, 1990. The Search Committee will begin screening dossiers on that date and will continue to review applications until the Vice President is selected.

Applications and nominations should be addressed to: **Search for Vice President for Research, Office of the President, The Ohio State University, 205 Bricker Hall, 190 North Oval Mall, Columbus, OH 43210.**

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Send curriculum vitae, one-page statement of research interests, representative reprints/preprints and three letters of reference postmarked by October 1st (or until a suitable candidate is found thereafter) to: Dr Robert J Spreitzer, Department of Biochemistry, University of Nebraska-Lincoln, Lincoln, NE 68583-0718. (402) 472-2932, FAX: (402) 472-7842.

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Interested individuals should submit a C.V. and bibliography, a one-page outline of research interests, and names and telephone numbers of three references to:

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Trinity College,
Dublin 2.

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to whom formal applications may be sent, *preferably before 5th October 1990.*

TRINITY COLLEGE IS AN EQUAL OPPORTUNITIES EMPLOYER

(4436)A

SYSTEMATIC BIOLOGY

University of Arizona

In the next phase of the development of an interdepartmental program in systematic biology, the University of Arizona invites applications for tenure-track positions; Assistant Professor in Insect Systematics and Assistant to full Professor in Plant Systematics. It is expected that the successful candidates will assume curatorial responsibilities, establish independent, externally funded research programs, and participate in graduate and undergraduate teaching. All specialties within systematics, broadly defined, will be considered, including field-oriented, collection-based, theoretical, and molecular approaches to problems at any taxonomic level. For example, systematists specializing in biogeography, conservation biology, evolutionary biology, pollination biology, or developmental biology, are encouraged to apply. The staff and facilities of the Molecular Systematics and Evolution Laboratory administered through the Center for Biotechnology will be available.

Candidates must have the PhD degree, and postdoctoral experience is desirable. A curriculum vitae, selected reprints, statement of research interests, and the names and addresses of at least three referees, should be submitted by 15th November, 1990.

INSECT SYSTEMATIST/CURATOR

This appointment will be in the Department of Entomology, with a possible adjunct appointment in the Department of Ecology and Evolutionary Biology. In addition to research and teaching of introductory and advanced courses in insect systematics, this position involves responsibility for the University's insect collection with 806,000 specimens, which is particularly strong in representation of the regional insect fauna. Curatorial experience is highly desirable.

Apply to Dr Elizabeth A Bernays, Head, Department of Entomology, University of Arizona, Tucson, AZ 85721.

PLANT SYSTEMATIST/CURATOR

This will be a split appointment in the departments of Ecology and Evolutionary Biology (principal department) and Plant Sciences. In addition to research and teaching, this position involves principal responsibility for direction of the University's herbarium, an outstanding collection of 414,000 specimens, primarily representing the arid Southwest. Curatorial experience is highly desirable.

Apply to Dr Michael J Donoghue, Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, AZ 85721.

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(NW5673)A

NEUROENDOCRINOLOGIST

The Neuroscience Unit at the Douglas Hospital Research Center is seeking a Ph.D., neuroendocrinologist with 1) a developed research program related to maturational changes in endocrine function, 2) a background in molecular and cellular endocrinology, and 3) an emphasis on hypothalamic-pituitary-adrenal function. The position is potentially tenure-track, depending upon the strengths and performance of the individual, who will be expected to develop a strong independent research program, acquire funding support, and participate in graduate student training. There are minimal teaching or administrative responsibilities. The Douglas Hospital Research Center is associated with McGill University and currently consists of 8 faculty members with research interests ranging from molecular biology to psychobiology, with a strong record of collaboration, as well as a number of graduate and post-doctoral fellows.

Send letter of interest, curriculum vitae, the names of three or more references and any inquiries to Michael J. Meaney, Douglas Hospital Research Center, Department of Psychiatry, McGill University, 6875 Boul, LaSalle, Montréal (Québec) H4H 1RS. Canada.

In accordance with Canadian immigration requirements, priority will be given to Canadian citizens and permanent residents possessing the required training and research background.

(NW5680)A

Leicester University

DEPARTMENTS OF PATHOLOGY AND MEDICINE

A Research Assistant or Research Associate

is required for a project investigating the neuroeffector junction in human resistance arteries in hypertension. The post is funded by the British Heart Foundation for three years. The study will involve the use of immunocytochemical and electron microscopic techniques to examine the innervation of the human microvasculature. The successful applicant will join an active smooth muscle research group dedicated to the investigation of cardiovascular diseases such as high blood pressure and will have the opportunity to register for a higher degree if desired. Previous experience would be preferred but is not essential because full training in the techniques can be provided.

Salary on the RA IA scale £11,399 to £18,165 pa. Starting salary will be up to £14,744, subject to age, qualifications and experience.

Further information can be obtained from Dr K.P. West (0533) 523221 or Dr A.M. Heagerty (0533) 523187.

Applications (including a c.v. and the names and addresses of two referees) should be sent to **Dr K.P. West, Senior Lecturer in Pathology, University of Leicester, Clinical Sciences Building, Leicester Royal Infirmary, Leicester LE2 7LX.**

Closing date for applications: 13th September, 1990. (4452)A

CHAIRPERSON

Department of Anatomy

The University of Nebraska College of Medicine is seeking a nationally-recognized investigator to fill the position of Chairperson of the Department of Anatomy. The candidate should have a PhD or MD degree, a distinguished research program that is currently active and funded, strong leadership qualities and a major interest in the education of health professionals. *Nominations or applications, including a curriculum vitae and the names and addresses of three references, should be addressed to:* **Chairman, Anatomy Chairperson Search Committee, c/o Office of the Dean, University of Nebraska College of Medicine, 600 South 42nd Street, Omaha, NE 68198-6545. Application deadline is October 1st, 1990.**

THE UNIVERSITY OF NEBRASKA IS AN EQUAL OPPORTUNITY/AFFIRMATIVE ACTION EMPLOYER

(NW5619)A

Q.M.W.

University of London

POSTDOCTORAL RESEARCH ASSISTANT

"Monoclonal Antibodies against Adrenocortical Cells"

Applications are invited for this MRC funded project based in the Department of Biochemistry within the brand new purpose built Faculty of Basic Medical Sciences at QMW. The appointment is for two years and will involve the identification and characterisation of antigens which may be involved in the regulation of steroid 18-hydroxylation and aldosterone production. Candidates should have completed PhD studies and have experience in the fields of monoclonal antibodies and/or molecular biology.

Salary will be on the RA1A scale up to a maximum of £15,805 per annum inclusive.

Application forms and further details are obtainable by telephoning 071-975 5171 (24 hours) quoting ref. 90/126. Alternatively send a full c.v. with the names and addresses of three referees to the **Personnel Officer (Recruitment), Queen Mary and Westfield College, Mile End Road, London E1 NS.** Closing date 13th September, 1990. (4434)A

UNIVERSITY of GUELPH

COLLEGE OF BIOLOGICAL SCIENCES

DEPARTMENT OF BOTANY

CHAIR

The University of Guelph is seeking a Chair for the Department of Botany. Candidates should have an outstanding record of research achievement and interest in promoting high standards in teaching at the undergraduate and graduate levels. The Department, with 15 full time faculty members, has research strengths in the areas of Physiology and Plant Molecular Biology, Ecology, and Plant Structure and Development. Faculty members also participate in the activities of a Plant Biology Program involving the Departments of Horticultural Science, Crop Science, and Environmental Biology. The Department offers undergraduate programs in Honors Plant Biology and Honors Ecology. Currently, 28 students are enrolled in graduate programs leading to M.Sc. and Ph.D. degrees.

The University, with a student population of 11,000 undergraduate and 1,400 graduate students, is located in the city of Guelph approximatley 80 km west of Toronto. The College of Biological Science, with 2,500 undergraduate majors and approximately 200 graduate students, is one of 7 Colleges in the University and has a faculty complement of 90 members. Research funding for the present year exceeds $7.5 million.

The University of Guelph is committed to an employment equity program that includes special measures to achieve diversity among its faculty and staff. We therefore particularly encourage applications from qualified aboriginal Canadians, persons with disabilities, members of visible minorities and women.

In accordance with Canadian Immigration requirements, priority will be given to Canadian citizens and permanent residents of Canada. This appointment is subject to final budgetary approval.

Interested candidates should submit a curriculum vitae, list of publications, and the names of three referees. Review of applications will begin on 30th October, 1990.

Dr. Bruce H. Sells, Dean
College of Biological Science
University of Guelph
Guelph, Ontario, Canada N1G 2W1

(NW5671)A

CHARING CROSS SUNLEY RESEARCH CENTRE

Arterial Disease Group

POSTDOCTORAL SCIENTIST

The Arterial Disease Group is an active group of 20 scientists with an international reputation for its work on the genetics of cardiovascular disease.

We have recently observed that proteases capable of degrading extracellular matrix are expressed by cells in atherosclerotic human coronary arteries, and thus may be involved in processes leading to acute thrombotic events. We are now looking for an enthusiastic postdoctoral scientist to work on a 3 year MRC project to study the control of expression of these and related genes in arterial tissues and cells, using molecular and cell biological techniques.

Experience in cell and molecular techniques would be an advantage. Those who have submitted, or are about to submit, their thesis are encouraged to apply.

Starting salary circa £15,262 inclusive of London Weighting, according to age and experience. Applicants should send a brief summary of their research interests, detailed cv and the names of two referees to Mrs J Brewer, The Charing Cross Sunley Research Centre, 1 Lurgan Avenue, Hammersmith, London W6 8LW.

Closing date: 13th September, 1990. (4433)A

NATIONAL UNIVERSITY OF SINGAPORE

Department of Physics

Applications are invited for

TEACHING APPOINTMENTS

in the Department of Physics. Candidates should possess a PhD degree in Physics and have teaching and research experience. Preference will be given to candidates specialising in Materials Science, Computational Physics and Experimental Nuclear Physics with experience in XRF/PIXE/RBS. A good knowledge of computing is essential and the successful candidate may also be required to teach computer languages and programming.

Gross annual emoluments range as follows:

Lecturer	S$50,390 - 64,200
Senior Lecturer	S$58,680 - 100,310
Associate Professor	S$88,650 - 122,870

(STG£1.00 = S$3.28 approximately)

The commencing salary will depend on the candidate's qualifications, experience and the level of appointment offered.

Leave and medical benefits will be provided. Depending on the type of contract offered, other benefits may include: provident fund benefits or an end-of-contract gratuity, a settling-in allowance of S$1,000 or S$2,000, subsidised housing at nominal rentals ranging from S$100 to S$216 pm, education allowance for up to three children subject to a maximum of S$10,000 per annum per child, passage assistance and baggage allowance for the transportion of personal effects to Singapore. Staff members may undertake consultation work, subject to the approval of the University, and retain consultation fees up to a maximum of 60% of their gross annual emoluments in a calendar year.

The Department of Physics is a department in the Faculty of Science. Its major research facilities include a 2.5 MV Van de Graaff accelerator, X-ray flourescence equipment, a JEOL SEM35CF scanning electron microscope, a VG ESCALAB MKII/AES + SIMLAB and several high-power tunable lasers. There are 8 faculties in the National University of Singapore with a current student enrolment of some 14,000.

All academic staff have access to the following computer and telecommunication resources: an individual microcomputer (an IBM AT-compatible or Apple Macintosh); an IBM mainframe computer with 16 MIPs of computing power; an NEC SX supercomputer with 650 MFLOPS of computing power; departmental laser printers; a wide spectrum of mainframe and microcomputer software; voice-mail; BITNET to access academic institutions world-wide. An on-line library catalogue is also available. In addition, a proposed campus network based on state-of-the-art optical fibre technology will be installed by the end of 1990 to facilitate resource sharing and electronic communication for the academic community.

Application forms and further information on terms and conditions of service may be obtained from: **The Director, Personnel Department, National University of Singapore, 10 Kent Ridge Crescent, Singapore 0511** *or* **NUS Overseas Office, Singapore High Commission in London, 5 Chesham Street, London SW1, UK.Tel: (071) 235 4562.**

Enquiries may also be sent through BITNET to: **PERPL @ NUS3090,** or through **Telefax: (65) 7783948.** (W7669)A

POSTDOCTORAL POSITION

Department of Biochemistry

University of Wisconsin-Madison

A postdoctoral position is available in the Department of Biochemistry at the University of Wisconsin-Madison for a joint project in Professors Julius Adler's and Bruce Selman's laboratories. The nature of the research to be pursued is the mechanism of sensory transduction in the eukaryotic green alga *Chlamydomonas reinhardtii.* In particular, the role of protein methylation will be examined in relation to the response of the alga to external stimuli.

Interested candidates should send their CV and arrange to have three letters of recommendation sent to either Prof Adler or Selman.

(NW5642)A

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§ IBPGR

IBPGR (International Board for Plant Genetic Resources) announces that it has begun a joint project with the International Development Research Centre (IDRC) to develop a guidebook/manual that will enable personnel in genetic resources programs to construct documentation systems that are tailored to their needs.

We are seeking a dynamic, innovative individual that is able to interact well, for long periods of time with people from varied cultural backgrounds and be able to work well for long periods of time away from their host country.

At least an MSc in the agricultural/biological sciences, the degree earned must represent experience in scientific experimental work. Experience in applying the personal computer in problem solving in the biological sciences, use of statistical and database management system software, and 3-5 years in the documentation of biological systems is a must. Extensive experience in teaching and training and the production of training matter in biological sciences is preferable.

The individual will be employed as a documentation associate based in Rome, Italy under the direction of the Documentation Coordinator. Salary will commensurate with experience.

Application: Potential candidates should write to the Director, IBPGR c/o Food and Agriculture Organization of the United Nations, Via delle Sette Chiese 142, 00145 Rome, Italy, Fax: 396-5750309, enclosing a full curriculum vitae and a list of 3 referees by 1st October 1990. (W7677)A

THEORETICAL NEUROBIOLOGY

The Neurosciences Institute (NSI) invites applications for positions as Institute Fellows in Theoretical Neurobiology. The NSI supports an in-house research program dedicated to developing experimentally testable theoretical models of neural systems. Current models include simulations of pharmacological effects on neuronal networks, the design of network automata, and analysis of visual systems. Advanced supercomputer facilities are available.

Institute Fellows are appointed for two years with the possibility of renewal and receive stipends that depend on qualifications and experience. Applicants should be at the postdoctoral or assistant professor level and must have a background in experimental or theoretical neuroscience or related fields.

The NSI is an independent organization located on the campus of The Rockefeller University in New York City. The NSI serves as the home of the Neurosciences Research Program and is an international center of study and research for distinguished visiting neuroscientists. Fellows have opportunities to use the institute's extensive facilities and to interact with visiting scientists.

Applicants should send a short statement of research interests, a curriculum vitae, and the names of three references to **Dr W. Einar Gall, Research Director, The Neurosciences Institute, 1230 York Avenue, New York, NY 10021.** (NW5665)A

MRC Clinical Research Centre
Watford Road
Harrow HA1 3UJ

MOLECULAR BIOLOGY OF PSYCHOTIC ILLNESS

Two scientific posts are available within the Division of Psychiatry at the Clinical Research Centre for work on the molecular genetics of psychiatric disease with particular reference to schizophrenia. Experience in linkage methods (including sibling pair analysis) or in in-situ hybridisation and other approaches to RNA in post-mortem brain tissue is relevant, but applications will also be considered from candidates with scientific or medical qualifications, some relevant experience and particularly with innovative approaches to this difficult problem. The appointments will be for 3 years and salary will be on the MRC scales appropriate to the candidates background and experience.

Enquiries should be directed to Dr T J Crow, Head of Division of Psychiatry, telephone number 081-869-3510. Application forms and further details are available from the Personnel Dept 081.869.3431 quoting ref: 125/1/4306. Closing date for completed applications is 14th September 1990.

The Medical Research Council is an Equal Opportunity Employer. (4424)A

Leicester University

DEPARTMENT OF BIOCHEMISTRY

Postdoctoral Research Associate

Sorting of Influenza Virus RNA

Applications are invited for an MRC-funded post to join a group investigating the mechanisms by which influenza virus transcripts enter different processing pathways. The work will involve mutagenesis, analysis of expression in mammalian cells, microinjection into oocyte nuclei and *in situ* hybridization with analysis by laser scanning confocal fluorescence microscopy.

The post is tenable for three years. Experience of interest in molecular biology or *in situ* analysis would be an advantage.

Salary on the RA IA scale £11,399 to £18,165. Starting salary will be up to £14,038, subject to age, qualifications and experience.

Applications (including a c.v. and the names and addresses of two referees) should be sent to **Dr Ian Eperon or Dr Chris Crane, University of Leicester, Leicester LE1 7RH,** as soon as possible. Enquiries can be made by telephoning (0533) 523482.

(4451)A

UNIDO

INTERNATIONAL CENTRE FOR GENETIC ENGINEERING AND BIOTECHNOLOGY

New Delhi Component

TWO POSTDOCTORAL POSITIONS

Plant and Insect Molecular Biology

Applications are invited for two postdoctoral positions in the Plant Molecular Biology Laboratory of ICGEB, New Delhi. The positions are funded for three years by a grant from the Rockefeller Foundation for research on the enhancement of insect resistance in rice with special reference to the gall midge. One post is for research on the transformation of rice with genes encoding insecticidal proteins; applicants should have experience with recombinant DNA techniques and plant transformation systems. The other post is for research on the molecular basis of genetic change and biotype formation in insect pests of rice; applicants should have experience with recombinant DNA techniques and entomology. Salaries are in the range US$15-20k.

Applications, with CV and names of 2 referees, should be sent to Mr G Chatterjee, Administrative Officer, ICGEB, New Delhi, NII Campus, Shaheed Jeet Singh Marg, New Delhi — 110067, India, by 31st October, 1990. Further information from Dr John Bennett (Tel: 91-11-6863184).

(W7675)A

MARIE CURIE RESEARCH INSTITUTE

NEW LABORATORY INITIATIVE: SENIOR INVESTIGATOR

Applications are invited for this senior post to establish a new laboratory as part of the expansion and development of this Institute. We support high quality basic and cancer-related research. This represents a unique opportunity for an innovative biologist with proven research ability to recruit and develop a new team. Funding for this initiative has been earmarked to support the salaries of the senior investigator, technical and postdoctoral staff as well as consumable and capital budgets. There is ample laboratory space and good central services support. Interaction with our existing groups is encouraged. Their interests include eukaryotic transcriptional regulation, cell cycle control/DNA replication, oncogene structure/function, molecular mechanisms of neurogenesis, herpesvirus gene regulation, etc.

Applications from suitably qualified candidates, including a CV and an outline of proposed investigation, should be sent to the Director, Dr Graham Currie, Marie Curie Research Institute, The Chart, Oxted, Surrey RH8 0TL, UK (Tel: (0)883 722306).

(4407)A

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proteus

PROTEUS INTERNATIONAL plc
PROTEUS MOLECULAR DESIGN LIMITED

Software Development Manager and Scientific Programmers

Several positions are available for Protein and Drug Molecular Modellers for both junior and senior positions. There is also an opening for a Software Development Manager and for at least three new positions for general scientific programmers. Staff will be concerned with the BioEngine™ project, either developing new design technologies, or using it to design new chemical products, either in-house or by joint collaborations with external organisations. The project demands willingness to work with exciting new approaches to molecular modelling and simulation, as well as the wide range of established methods. Software technologies include the GLOBAL™ high level language for chemists and molecular biologists, accessing the PROMETHEUS™ expert system for molecular modelling, and novel types of dynamics and neural net technologies. Hardware includes a Convex 220, Silicon Graphics, IBM and transputer equipment with linked access to Cray and IBM Supercomputers. Excellent opportunities exist for R&D collaboration with major European universities and American hardware and biotechnology companies. Participation in basic research and scientific publication is encouraged.

Applications with CV to Mr John K Pool, Managing Director, PROTEUS INTERNATIONAL plc, PROTEUS HOUSE, 48 Stockport Road, Marple, Cheshire SK6 6AB. Tel: (061) 426 0191, Fax (061) 426 0192.

(4442)A

JCU JAMES COOK UNIVERSITY OF NORTH QUEENSLAND

FIXED TERM LECTURER

Sedimentology/Marine Geoscience

(Re-Advertisement)

Applications are invited for the position of Fixed Term Lecturer in Sedimentology/Marine Geoscience within the Department of Geology. The position will be available for a period from January 1991 to 31 December 1993, and may be renewed thereafter.

Applicants must have, or be near completion of, a PhD on some aspect of sedimentology, applied sedimentology or sedimentary marine geoscience. Microcomputer experience and a strongly developing publication record are highly desirable. Other desirable experience would include expertise in hydraulics of sediment transport, or environmental geoscience, and direct experience of marine fieldwork (seismic-sidescan survey, vibrocoring, etc., or environmental science).

The appointee will join an active and experienced research group in sedimentary geology and geophysics. Current research encompasses outcrop, and modern shelf and deep-ocean studies, including GLORIA survey. The University offers great strength in marine science studies, and strong research facilities, including modern analytical equipment (SEM-TEM, microprobe, XRD, XRF), field equipment (land and marine drill rigs), marine survey equipment (20m vessel, PDR, sidescan) and excellent microcomputer and workstation facilities.

Further information on the duties of the position may be obtained from Professor R.M. Carter on 077/814536 or facsimile 077/251501.

Salary range for the position is A$33,163 – A$43,096 a year and commencing salary will be determined in accordance with qualifications and experience.

Further information and application forms may be obtained from the Personnel Manager, James Cook University, Townsville Qld 4811, Australia. Applications close on 28 September 1990. In reply please quote reference number N90148.

Equal Opportunity in Employment is University Policy.

(W7673)A

UNIVERSITY OF BRISTOL — LECTURER IN ANATOMY

(with special responsibility for dental students)

The Department of Anatomy at Bristol has responsibilities for students in the Medical, Dental and Veterinary Schools as well as the Faculty of Science. The duties of the present post are mainly concerned with teaching anatomy and oral histology to dental students but will extend into other areas in relation to the successful candidate's expertise. There are excellent research facilities in the department, with active research groups in Oral Biology, Comparative Orthopaedics, Neurobiology and Reproductive Biology. A dental qualification is not essential but would be an advantage. Informal telephone enquiries will be welcomed by Professor B T Pickering (0272 303488).

Applications, in duplicate, including curriculum vitae and the names of three referees, should be lodged with the Registrar, University of Bristol, Tyndall Avenue, Bristol BS8 1TH by 30th September 1990 from whom further particulars may be obtained. Please quote reference JPB/Den/22. (4431)A

UCL

UNIVERSITY COLLEGE AND MIDDLESEX SCHOOL OF MEDICINE

DEPARTMENT OF PHYSIOLOGY

Post-Doctoral Research Assistant (Grade 1A)

A vacancy exists for a suitably qualified candidate in a new group investigating protein phosphorylation in the cell cycle. The position is funded by a grant from the Leukaemia Research Fund for three years, and focuses on signal transduction mechanisms that regulate T-lymphocyte proliferation. Some of the studies will be performed in collaboration with Dr. Doreen Cantrell of the Imperial Cancer Research Fund.

Apply in writing, enclosing two copies of your c.v. to Dr Stephen E. Moss, at the Department of Physiology, University College London, Gower Street, London WC1E 6BT. The starting salary according to age and experience £16,000-£17,500, and the position is available immediately. Tel: 071-387 7050, ext. 3250.

Equal Opportunities Employer. (4454)A

SCHOOLS OF CHEMICAL AND BIOLOGICAL SCIENCES

CENTRE FOR METALLOPROTEIN SPECTROSCOPY AND BIOLOGY

UEA NORWICH

Research Associate in Enzymology and Spectroscopy of Cytochrome o.

Applications are invited for a post-doctoral research associateship (on the Research and Analogous 1A salary scale ranging from £11,399 to £18,165 per annum according to age and experience, funded by SERC) to investigate the structure and mechanism of action of the ubiquinol oxido-reductase of E.coli which contains cytochrome and copper centres. The enzyme, expressed by an overproducing strain of the organism will be studied by a variety of spectroscopic methods developed at the SERC Molecular Recognition Centre.

Applications, including the names of three referees, should be sent as soon as possible either to: Professor A. J. Thomson, School of Chemical Sciences, of Professor C. Greenwood, School of Biological Sciences, University of East Anglia, Norwich NR4 7TJ, United Kingdom, from whom further information can be obtained. Tel: (0603) 56161, ext. 2023 or ext. 2196. Fax: (0603) 58553.

UEA is an equal opportunities employer.

(4446)A

UNIVERSITY COLLEGE LONDON

Department of Anatomy & Developmental Biology and Department of Biochemistry

Postdoctoral position in Developmental Biology

Applications invited for a 3 year position to study "The role of homeobox genes in patterning of developing vertebrate limbs". The position is available from October 1, 1990 but a later starting date is possible.

Salary up to £13,495 pa (plus £1,767 pa London Allowance) depending on qualifications and experience.

Send c.v. and names of 2 referees to Dr C. Tickle, Department of Anatomy and Developmental Biology, UCMSM, Windeyer Building, Cleveland Street, London W1P 6DB or phone 071-636 8333, ext. 3135 for further details.

Equal Opportunities Employer. (4456)A

LONDON SCHOOL OF HYGIENE & TROPICAL MEDICINE

POST-DOCTORAL SCIENTIST

Department of Clinical Sciences

Applications are invited for the post of post-doctoral scientist for a three year MRC Aids Directed Programme sponsored project on virus-antibody interactions in which characterization and the biology of HIV-IMMUNE COMPLEXES are studied by virological, molecular-biological (including PCR), biochemical and immunological techniques.

Applicants should send a full curriculum vitae and the names of two referees to Ms Helen Abbott, Personnel Officer, LSHTM, Keppel Street, London WC1E 7HT, quoting reference KT3.

Salary up to £12,381 plus £1,767 London Weighting (under review). Telephone 071 636 8636, ext. 203.

Closing date: 10th September, 1990.

(4425)A

Royal College of Surgeons in Ireland

PROFESSOR/ASSOCIATE PROFESSOR OF CLINICAL PHYSIOLOGY

Applications are invited for the above post at the Royal College of Surgeons in Ireland.

Candidates for the post should possess:-

(i) An appropriate postgraduate qualification, e.g. Fellowship, Membership, Ph.D.,or equivalent and a record of substantive academic achievement.

(ii) Previous experience of teaching medical students would be an advantage.

(iii) The College is conscious of a need to encourage development in Clinical Physiology in the Department, therefore, preference may be given to applications from those candidates who are medically qualified.

Further particulars may be obtained from:-

The Chief Administrator/Academic Secretary,
Royal College of Surgeons in Ireland,
123, St. Stephen's Green, Dublin 2, Ireland.
Telephone: 780200 (ext. 292)

to whom curriculum vitae (15 copies) should be sent. Closing date for receipt of applications is **Friday 28th September, 1990.** (4437)A

THE INTERNATIONAL RICE RESEARCH INSTITUTE

seeks a

PLANT PHYSIOLOGIST

The International Rice Research Institute, is an international, donor funded non-profit, agricultural research institute that undertakes research and training at its headquarters in the Philippines and collaborates with national agricultural systems worldwide. Its goal is to enhance rice production and sustainability to benefit the world's 1.3 billion people who are dependent on rice for their livelihood and as their basic food. IRRI seeks a Plant Physiologist who will work with interdisciplinary teams of scientists such as plant breeders, geneticists and soil scientists.

Responsibilities:

- Provide disciplinary leadership and implement research projects on nutrient dynamics, methods for evaluating useful physiological traits, and crop canopy photosynthesis in different ecosystems, plant types and methods of crop establishments as they relate to increased grain yield potentials of rice in different ecosystems.
- Contribute to IRRI's program of degree and non-degree training. This includes serving as adviser to MS and PhD. students.
- Work with national agricultural systems in conducting practical and strategic research to solve regional or local problems.

Qualifications:

- Ph.D. in plant or crop physiology.
- Experience in tropical agriculture highly desirable.
- Expert knowledge in plant nutrition and photosynthesis is important.
- Excellent ability to speak and write English is required. Published or accepted papers in international journals are desirable.
- International travel is required.

Benefits:

Salary and perquisites will be commensurate with those of similar international institutions and programs in agriculture. The person chosen for the position will live at IRRI headquarters on the campus of the University of the Philippines at Los Banos, 60 km south of Manila.

Application:

Applications with full documentation, including three referees, and names of nominees should be addressed to: Dr. Klaus Lampe, Director General, IRRI, PO Box 933, 1099 Manila, Philippines.
Tel. No. (63-2) 818-2088. Fax: 63-2-817-2087.
Telex: 40890 RICE PM or 22456 IRI PH.

Interviews of short-listed applicants will commence in September, 1990. (W7674)A

UNIVERSITY OF OXFORD

(AN EQUAL OPPORTUNITY EMPLOYER)

MCDONNELL-PEW CENTRE FOR COGNITIVE NEUROSCIENCE

An award from the McDonnell-Pew Program in Cognitive Neuroscience, based in the United States of America, has funded the establishment of a Centre for Cognitive Neuroscience at the University of Oxford. The Centre, links work on many aspects of brain research relevant to human cognition in several departments at Oxford and other institutions. The Centre invites applications for the following positions, which will be made for two years in the first instance, with the possibility of renewal for a third year.

Postdoctoral Research Fellowships

Three Research Fellowships on the RS1A scale (£11,399 to £18,165 per annum, discretionary range to £20,372, depending on age and experience) are offered for experienced scientists to work in collaboration with existing research groups. These appointments are intended to stimulate the application of neural network or other computational modelling methods to neurophysiological and psychological studies of perceptual analysis and memory, and the linking of basic psychophysical and behavioural research with clinical work on spatial perception and memory. Applicants should have a good background in research in one of the following fields:

Computational neuroscience
Cognitive psychology
Neuropsychology

Computer Officer

Applications are invited from individuals with extensive experience of modern computing techniques for the post of Computer Officer to the Centre, to be appointed on the RSII scale (£16,755 to £22,311 per annum, discretionary range to £24,172, depending on age and experience). The holder of this post will advise on and assist with computing activities for the Centre (real-time experimental control, data analysis, computational modelling, etc) and will co-ordinate software, interfacing and other hardware development for particular research projects. A budget of about $40,000 per annum will be available for the purchase of essential supplies and the sub-contracting of some programming and hardware development.

An **ADMINISTRATIVE SECRETARY** will be appointed on the Grade 5 scale (£11,244-£13,202 per annum, discretionary range to £14,557, depending on age and experience) to run an office, from which the activities of the Centre will be co-ordinated. Applicants for this challenging position should be capable of working on their own initiative and must have excellent organizational ability, as well as basic secretarial and accounting skills.

Applications for all these posts, including the names and addresses of two referees and a full curriculum vitae should be sent to the Administrator, University Laboratory of Physiology, Parks Road, Oxford OX1 3PT, by 21st September, 1990. Further information can be obtained from Professor Colin Blakemore at the same address. Interviews will be held as soon as possible with a view to appointments being made in October. (4465)A

SOUTH EAST THAMES REGIONAL GENETICS CENTRE

CYTOGENETICISTS

GRADE A OR GRADE B

This is a busy centre, providing pre and post-natal screening for chromosome disorders. Cytogenetics at Guy's Hospital is an integral part of the Division of Medical and Molecular Genetics, which embraces a range of disciplines, including biochemical genetics and recombinant DNA work.

For the Grade B post you should have several years' experience in human cytogenetics and will be expected to take a responsible role in helping to run the diagnostic services.

For the Grade A post you should have a good honours degree in genetics or another biological subject. Previous experience in cytogenetics is always welcome, but not essential and full training will be given in all aspects of the work, which includes tissue culture and microscopy.

Further information may be obtained from Dr Michael Drake or Dr Zoe Docherty on 071-955 4448 or 071-955 5000 ext 2542 respectively.

For application papers please contact the Personnel Department, Guy's Hospital, St Thomas Street, London SE1 9RT. Tel: 071-955 5000 ext 5092/3 quoting reference number P/92.

Closing date: 7th September, 1990.

Aiming to be an Equal Opportunities Employer. (4441)A

BRITISH HEART FOUNDATION

LECTURER/SENIOR LECTURER BASIC SCIENTIST RESEARCH POSTS

The British Heart Foundation invites applications from post-doctoral research workers to compete for a number of basic scientist research posts (non-clinical) at lecturer or senior lecturer level. The research programme need not necessarily relate to cardiology, but should relate to the cardiovascular system in its broadest sense and be undertaken in a university department within the UK.

An award would be for a period of five years with the possibility of an extension of not more than a further five years. The award reimburses for salary at the appropriate university scale and carries an allowance of up to £10,000 per annum for research expenses.

Applicants must be UK citizens, or have worked in the UK for at least the past three years, or must have done so before taking up an appointment overseas. The candidate should be aged approximately 28-33 years and should have shown outstanding ability in carrying out original and independent research.

Application may be made by the head of department or the candidate with support from the head of department.

Application forms and further details (quoting reference N/BS) are available from:

Valeria Mason
The Research Funds Manager
British Heart Foundation
14 Fitzhardinge Street
London
W1H 4DH

Closing date for applications: 13 December 1990

(4463)A

DEPARTMENT HEAD

ECOLOGY, ETHOLOGY, AND EVOLUTION

UNIVERSITY OF ILLINOIS

The University of Illinois at Urbana-Champaign invites nominations and applications for the position of Head of the Department and Professor of Ecology, Ethology, and Evolution. The department has 12 faculty and 12 affiliated faculty. The administration has made a strong commitment to further development of population biology and several new openings exist for recruitment of faculty members. Collaborative research opportunities are possible with the State Natural History Survey, Institute for Environmental Studies, Museum of Natural History, other departments in the School of Life Sciences, and departments in the Colleges of Agriculture and Veterinary Medicine. Campus facilities and resources available to the department include several nearby natural areas, including forest, restored prairie, and old-field habitats, collections in the Museum of Natural History, Herbarium, and State Natural History Survey, greenhouses, a National Center for Supercomputing Applications, a Biotechnology Center offering services for recombinant DNA technology and protein sequencing, and a Center for Genome Analysis.

We seek a person who holds a PhD degree, has a distinguished, active research program in an area of population biology, such as theoretical population biology, population genetics, or behavioral ecology and has demonstrated leadership qualities.

Submit nominations or applications with curriculum vitae and names and phone numbers of three references to: **Dr Carol K Augspurger, Chair, EEE Department Head Search Committee, University of Illinois, 393 Morrill Hall, 505 S Goodwin, Urbana, IL 61801. Tel: 217-333-1298, Fax: 217-244-1224.**

In order to ensure full consideration, applications must be received by 1st October, 1990. Salary is negotiable. Preferred starting date is 21st August, 1991.

The University of Illinois is an Affirmative Action/Equal Opportunities Employer.

(NW5645)A

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- to save time and for your added convenience you can contact us by fax.
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CELL BIOLOGIST

Merck Sharp & Dohme Research Laboratories is offering an excellent career opportunity in the Department of Virus and Cell Biology for an endothelial cell biologist at the research facilities in West Point, PA, located 25 miles northwest of Philadelphia, PA.

The successful candidate for this position will play a leading role in a multi-disciplinary project involving the regulation of coagulation by the endothelium.

The position requires a Ph.D. or M.D. degree and three (3) years experience in manipulation of primary cultures of endothelial cells.

Excellent salary and benefit programs accompany this position.

Please send curriculum vitae with cover letter to: **Personnel Manager, Ad #B-28, Research Personnel, WP42-2, Merck Sharp & Dohme Research Laboratories, West Point, PA 19486.** (NW5662)A

EEO/AA/VH/Employer

Merck & Co., Inc.

Biopolymers Facilities Director

The Howard Hughes Medical Institute Unit at the Harvard Medical School is seeking a qualified individual to supervise a modern biopolymers laboratory. This facility provides solutions to specific synthetic and degradive problems involving protein sequencing and peptide/nucleic acid synthesis. Ideal candidates should be experienced with various purification techniques, gas phase protein sequencing and automated nucleotide and peptide synthesizers. Emphasis will be given to those who have a demonstrated background managing such a facility.

This immediate opening represents an important service and development/research role with ample opportunity for growth. The successful candidate must be able to interact productively with a variety of colleagues on a problem solving basis.

Interested individuals should submit a curriculum vitae, a brief letter summarizing their qualifications and ask that three individuals send letters of reference. These materials should be sent to:
Dr. Philip Leder, Senior Investigator, Howard Hughes Medical Institute, Department of Genetics, Harvard Medical School, 25 Shattuck Street, Boston, MA 02115.

We are an Equal Opportunity Employer

Howard Hughes
Medical
Institute (NW5676)A

Protein/Biomolecular NMR Spectroscopist

Strong growth in the areas of molecular genetics and peptide research has created exceptional opportunity with Pfizer Inc, a Fortune 100 multinational leader in pharmaceutical research and healthcare products.

In this position, you will be responsible for equipping and launching a new laboratory dedicated to biomolecular problems. As part of a high-profile multidisciplinary team supporting discovery efforts within Central Research, you will work with instrumentation that consists of seven high field NMR spectrometers ranging from 250-500 MHz. Your investigations into drug structures/interactions with proteins and other macromolecules in solution will be the basis for the development of molecular modeling as a tool for drug discovery.

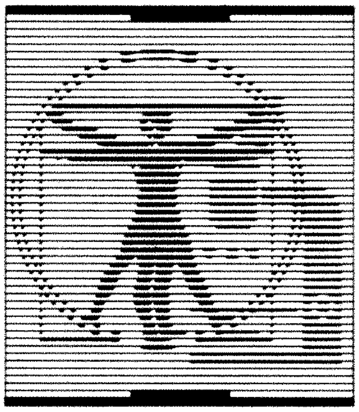
To qualify, you must have a PhD and postdoctoral experience in the application of NMR to structural studies of proteins and other biomolecules. Requires a strong fundamental background in NMR, knowledge of higher level computer programs for solving structure problems, familiarity with molecular modeling needs, and expert communication skills.

We offer a stimulating professional environment in an attractive Connecticut shore area complemented by a competitive salary/benefits package. If qualified, please submit a detailed resume with salary history/requirements in confidence to: Mr. Charles P. Norris, Supervisor Employee Resources, Central Research Division, Pfizer Inc, Eastern Point Road, Groton, CT 06340. An equal opportunity employer.

Pfizer

(NW5667)A

BIOMEDICAL RESEARCH



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At the forefront of biomedical research, Program Resources, Inc. (PRI) is paving the way for new programs and discoveries at the National Cancer Institute's Frederick Research and Development Center (NCI-FCRDC). Currently, we are seeking **SCIENTISTS/TECHNICIANS** with expertise in:

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BIOCHEMISTRY FACULTY POSITIONS

University of Wisconsin-Madison

The University of Wisconsin-Madison Biochemistry Department invites applications for two tenure-track positions at the Assistant or Associate Professor level. We are particularly interested in applications from individual whose research emphasizes METABOLIC REGULATION (Assistant or Associate Professor level) or MOLECULAR NEUROBIOLOGY (Assistant Professor level); however, outstanding candidates in any area of biochemistry will be considered. The successful candidates are expected to develop vigorous independent research programs and participate in the undergraduate and graduate teaching of general and specialized biochemistry courses.

Applications, including curriculum vitae, list of publications, summary of past work and accomplishments, and a statement of plans for future research, should be sent to Professor William S Reznikoff, Chairman, Department of Biochemistry, University of Wisconsin-Madison, 420 Henry Mall, Madison, WI 53706 USA.

In addition, applicants should arrange for at least three letters of reference to be mailed to the same address. Applicants should state in their cover letter for which position they wish to be considered. To assure consideration, all application materials should be received by October 1st, 1990.

The University of Wisconsin-Madison is an Affirmative Action, Equal Opportunity employer, and encourages applications from women and minorities. (NW5630)A

The Department of Chemistry of The University of Chicago

invites applications from outstanding individuals for

ASSISTANT PROFESSOR (TENURE TRACK) FACULTY POSITIONS

in inorganic, organic, physical, and polymer chemistry. Postdoctoral experience is preferred.

Applicants should submit a curriculum vitae, a list of publications, a succinct outline of their research plans and should arrange for three letters of recommendation to be sent to **Graham R Fleming, Chairman, Department of Chemistry, The University of Chicago, 5735 S Ellis Ave, Chicago, IL 60637.** An Equal Opportunity/Affirmative Action Employer. (NW5648)A

MOLECULAR MICROBIAL ECOLOGY

Institute of Virology & Environmental Microbiology, Oxford

Applications are invited from highly motivated individuals with a consuming passion for either Microbial Ecology, Gene Transfer in the Natural Environment or Prokaryotic Genetics. The available projects include the development of marking and monitoring techniques for the environmental detection of bacteria, investigations into the diversity, distribution, dispersal and persistence of microbes on plant surfaces and evaluation of gene flow in bacterial communities. Experience in one of the above would be an advantage.

Starting salary will be in the range of £10,678-£14,909 (Higher Scientific Officer level) or £9,131-£13,262 (Scientific Officer level). Additional pay award due August 1990. There is a non-contributory pension scheme. All appointments will initially be for a fixed term.

Further information is available from Dr M Bailey or Dr I Thompson at the Institute of Virology and Environmental Microbiology, Mansfield Road, Oxford OX1 3SR. Tel: (0865) 512361. Requests for application forms should be made to the Administration Officer at the above address.

The Natural Environment Research Council is an equal opportunity employer.

**Natural
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Council**

(4422)A

SALFORD HEALTH AUTHORITY

ROYAL MANCHESTER CHILDREN'S HOSPITAL

WILLINK BIOCHEMICAL GENETICS UNIT

TOP GRADE BIOCHEMIST (GRADE C)

(£22,771 p.a. to £31,164 p.a.)

The Unit is the Regional Amino Acid Reference Centre and provides extensive services for a wide range of inherited metabolic disorders on a Regional or Supra-Regional basis. The post is unique in the UK and requires a combination of scientific and managerial skills. Research will also be encouraged. Experience in the field of inborn errors of metabolism or related disciplines would be an advantage.

The successful applicant will be responsible for satisfactory running of all biochemical services provided by the laboratory. The appointee will also be responsible to the Director of the Unit.

For further information or to arrange an informal visit contact Dr I.B. Sardharwalla on 061-794 4696, ext. 2137/8.

An application form and job description can be obtained from the Personnel Department, Royal Manchester Children's Hospital, Pendlebury, Manchester M27 1HA on 061-727 8312 (answerphone).

Quote ref: RM03. Closing date: 7th September, 1990.

Salford Health Authority, an Equal Opportunities Employer for all, irrespective of sex, marital status, race, religion, creed, colour, disability or age. (4462)A

NOTTINGHAM HEALTH AUTHORITY

CITY HOSPITAL

CLINICAL CYTOGENETICIST SECTION HEAD

Postnatal Diagnosis Section Head in this busy, well established sub-Regional Clinical Cytogenetics Department required. Candidates should have extensive experience of diagnostic Cytogenetic work, and preferably hold the DipRCPath qualification.

Salary: B-Grade range £17,996 to £23,682 (under review).

Informal enquiries and visits to the Department: Contact Dr Tony Parkin (0602) 627617.

Application forms and job descriptions from the Personnel Department, Unit Headquarters, City Hospital, Hucknall Road, Nottingham NG5 1PB. Tel: (0602) 627672 (24 hour answering service).

Closing date: 7th September, 1990. (4439)A

UNIVERSITY OF MANCHESTER

Department of Chemistry

Research Associate in Protein Crystallography

A post-doctoral position, funded by the Wellcome Trust, is available to study the trypanosounal enzyme, trypanothione reductase. The characterisation of enzyme-substrate and enzyme-inhibitor complexes will also be undertaken. Experience in protein crystallography is desirable. The position is funded for up to 5 years with a starting salary of not less than £12,792 pa. Informal enquiries to Dr Bill Hunter (061 275 4712). Applications, including a detailed c.v. and names of 2 referees should be forwarded to Dr W.N. Hunter, Department of Chemistry, the University, Manchester M13 9PL.

The University is an Equal Opportunity Employer. (4457)A

POST-DOCTORAL CELL BIOLOGIST

Through a newly established Link Project between the AFRC Institute of Animal Physiology and Genetics Research at Babraham, the University of Cambridge Department of Veterinary Clinical Studies and the T.B.A. Equine Fertility Unit in Newmarket, a vacancy exists for an enthusiastic and hard-working person who has knowledge, expertise and interest in the areas of tissue culture and cellular/molecular biology.

The successful applicant will play the leading role in a collaborative study of factors that govern the growth and differentiation of trophoblast, and which influence the endocrinological and immunological interactions between mother and fetus during early pregnancy in the mare. This is an exciting opportunity for an ambitious young scientist to delve deeply into an interesting area of cell function in a pertinent and fascinating animal species.

He or she will function both at Babraham and Newmarket and will have a comprehensive range of laboratory equipment and animal handling facilities at their disposal.

Salary within the University Post Doctoral Research Associates scale, £11,399-£16,755 pa.

Telephone Dr W.R. Allen (0638) 662491, or Dr Francesca Stewart (0223) 832312 for further details, or send a written application, plus c.v. and the names of two scientific referees, to: The Secretary, T.B.A. Equine Fertility Unit, Mertour Paddocks, Woodditton Road Newmarket, Suffolk CB8 9BH Fax: 0638-667207. (4459)A

SENIOR FERMENTATION SCIENTIST

the discovery and development of new therapeutic agents

ICI Pharmaceuticals is one of ICI's most successful international businesses and has an outstanding record of achievement and innovation. Scientific talent and heavy investment in advanced biotechnology resources have combined to produce new therapeutics for the treatment of human disease.

We now have an exciting opening for a senior PhD scientist with a track record of achievement in large scale animal cell culture to lead a team working within the Fermentation Group of our Biotechnology Department. This group works within multi-disciplinary research and development teams, in close collaboration with pharmacologists, molecular biologists and protein chemists to identify and produce biologically active proteins and cell lines for research and clinical evaluation.

This is a first class opportunity to develop a career in an area of Biotechnology which is achieving remarkable results at the forefront of scientific research.

An attractive salary is offered and benefits include pension, private healthcare, profit share and relocation assistance to this attractive part of the country ... close to the Peak District and only 18 miles south of Manchester.

Write with c.v. and salary details, to:
The Personnel Officer, RCH/AMS,
ICI Pharmaceuticals, Mereside, Alderley Park,
Nr Macclesfield, Cheshire SK10 4TG.

(4464)A

ICI Pharmaceuticals

CELL BIOLOGY, MOLECULAR BIOLOGY, PROTEIN PURIFICATION

Bone Development, Regeneration and Repair

Chiron Corporation, a leader in soft tissue growth factor and wound healing research is expanding its growth and differentiation factor program in the area of bone morphogenesis.

We seek a motivated and dedicated scientist at the Ph.D. level to investigate the role of various novel recombinant bone proteins in the morphogenesis and repair of bone. Particular emphasis will be placed on bone repair models. The role of bone morphogenetic proteins in diseases associated with abnormal bone resorption will also be targeted.

Applicants should have at least two years of post-doctoral experience in bone cell biology or related fields. Also, candidates with some background knowledge of the molecular biology of bone would be preferred. An ability to perform research independently must be demonstrated by a record of publication in peer reviewed journals.

Two other positions exist at the B.S. or B.A. level for Research Associates with an interest in bone proteins at the molecular level. The first involves the molecular cloning, expression and post-translational processing of novel bone-related proteins. The second position will involve the purification of recombinant bone proteins produced in heterologous microorganisms such as yeast, or in cultured mammalian cells. For these latter two postions, entry level applications are encouraged.

Chiron Corporation, located in the San Francisco Bay Area, offers a stimulating and challenging research environment, a competitive compensation package and opportunities for professional advancement. Candidates please send C.V. and 2 references to **Chiron Corporation, Human Resources Dept. - MB148-49, 4560 Horton St., Emeryville, CA 94608.**

CHIRON
CORPORATION

(NW5654)A

THE UNIVERSITY OF SHEFFIELD

KREBS INSTITUTE FOR BIOMOLECULAR RESEARCH

TWO POST-DOCTORAL RESEARCH WORKERS AND ONE TECHNICIAN

Required immediately for 3 years for a major new LINK programme jointly funded by the SERC, DTI, Celltech Ltd and Roche Products to carry out fundamental studies on the isolation, structure, mechanism and improvement of catalytic antibodies, building on recent developments in the isolation and expression of antibody fragments in bacterial systems.

Based in the Departments of Molecular Biology & Biotechnology and Chemistry within the Krebs Institute, the programme will be directed by Dr D Burton and Dr M Blackburn. Candidates with experience in other relevant disciplines are encouraged to apply.

Research Post (Ref R1045/A) Required to construct libraries of PCR amplified antibody sequences, to screen them for catalytic antibodies and to engineer such antibodies for improved properties. A molecular biology post for which experience in DNA manipulation and/or antibody engineering is preferred.

Research Post 2 (Ref R1045/B) Responsible for characterisation and structure/function studies on catalytic antibodies. Experience in protein chemistry and/or enzymology preferred.

Technician (Ref R1045/C) Required to give technical support to the research staff. Ideally a graduate or HNC, experience in a biochemistry, microbiology or immunology laboratory is preferred.

Research Staff salary on Grade 1B or 1A (£11,399 — £18,165 pa) according to age and qualifications. Technician salary on Grade C or D (£8,405 — £11,589 pa) according to qualifications and experience.

Informal enquiries to Dr A Mountain, Celltech Ltd (0753 34655, ext. 2381). Applications (2 copies), including CV and the names/addresses of two referees should be sent to the Director of Personnel Services, The University of Sheffield S10 2TN by 13th September, 1990. Please quote the appropriate reference number.

AN EQUAL OPPORTUNITY EMPLOYER

At the **Swiss Federal Institute for Water Resources and Water Pollution Control (EAWAG)** in Dubendorf, Zurich, the post of a

DIRECTOR

will be vacant from 1st April, 1992, on the retirement of the present holder.

The EAWAG is a multi-disciplinary research institute (staff: ca. 250) of the Swiss Confederation, with intensive international contacts, reporting to the Board of the Swiss Federal Institutes of Technology Zurich and Lausanne. Its current research activities include chemistry and analytical chemistry; technical biology; engineering sciences; hydrobiology and limnology; fisheries science; waste and materials management; environmental physics; multi-disciplinary limnological research and earth sciences. The research institute is well equipped with a technical infrastrucutre, and participates in teaching at the ETH Zurich. It also organises symposia on aquatic ecology, atmospheric science, air pollution, sewage purification and waste management, and enjoys close contact with comparable scientific institutes in Switzerland and abroad.

The Director in charge of the EAWAG is responsible for the smooth running of all operations, scientific, technical, and administrative. His or her wish to be engaged in teaching at the ETH Zurich or Lausanne is an option we welcome.

We are looking for a mature personality who can handle this challenging task, one who knows from experience how to lead a team either in university or industrial context. The applicant's prerequisites are: university training, wide-ranging and successful research activities in areas such as chemistry, biology, physics, environmental or engineering sciences — coupled with a good command of German, French and English.

Applications should be addressed to the President of the Board of the Swiss Federal Institutes of Technology, ETH-Zentrum, CH-8092 Zurich, no later than 31st October, 1990. (W7667)A

UNIVERSITY OF SOUTHAMPTON

Lymphoma Research Unit, Tenovus Laboratory, General Hospital, Southampton SO9 4XY

A POSTDOCTORAL SCIENTIST

is required to participate in a long-term programme studying the killing of neoplastic lymphocytes by antibody, with subsidiary projects on lymphoma surfaces and antibody effector mechanisms. There will be an opportunity to participate in a current clinical trial of the treatment of lymphoma with antibody derivatives. Salary on University 1A scale for research staff (£11,399-£18,165 pa). Appointment for 3 years with possibility of extension.

Enquiries to Professor G T Stevenson at the above address.

Applications to include 2 copies of cv and the names and addresses of 2 referees to Reference M/17, Staffing Department, University of Southampton, Highfield, Southampton SO9 5NH, by 10th September 1990.

WORKING FOR EQUAL OPPORTUNITIES (4458)A

Genetics, Cell and Molecular Biology, and Development

Department of Biochemistry, Baylor College of Medicine

The Department of Biochemistry has

TWO TENURE TRACK FACULTY POSITIONS

in the areas of genetics, cell and molecular biology, and development. These positions provide recently renovated laboratory space, institutional start-up funding, limited teaching requirements, and an excellent opportunity to build a research program within a large and interactive research community. Applicants at the assistant professor level are preferred, but more senior candidates will be considered.

Interested individuals should send their curriculum vitae, a statement of present and future research interests, and the names of three references to: **Dr John Wilson, Search Committee, Department of Biochemistry, Baylor College of Medicine, One Baylor Plaza, Houston, Texas 77030.**

An Equal Opportunity Employer.

(NW5652)A

RESEARCH ASSISTANT PROFESSOR

Responsibilities include research and teaching. Position is available for PhD with three or more years of post-doctoral experience. Expertise using cell and molecular biological approaches to study receptor-coupled gene expression in smooth muscle is preferred.

Send CV and three letters of reference to: **M M Grunstein, MD PhD, Division of Pulmonary Medicine, Children's Hospital of Philadelphia, University of Pennsylvania, 34th Street and Civic Center Blvd, Philadelphia, PA 19104.**

The University of Pennsylvania is an equal opportunity/affirmative action employer.

(NW5685)A

POSTDOCTORAL SCIENTIST

Harvard Medical School

Position available immediately for study of the erythropoietin (EPO) receptor and signal transduction. Experience in hematopoiesis research and molecular biology is advantageous, but highly motivated individuals with a background in related areas are encouraged to apply. Send c.v. and the names of three references to: **Dr Alan D'Andrea, Dana Farber Cancer Institute, 44 Binney Street, Boston, Massachusetts 02115, USA. Tel: 617-732-3537.**

(NW5659)A

POSTDOCTORAL POSITIONS

available to study the regulation of the intracellular phospholipases that mediate stimulus-response coupling in mammalian cells. Projects include delineation of the mechanisms responsible for the activation of intracellular phospholipases A_2 as well as studies directed toward their cloning, expression, site-directed mutagenesis and intracellular processing. Candidates should be recent PhDs with experience in protein chemistry, lipid chemistry or molecular biology.

Send curriculum vitae and the names of two references to: **Richard W Gross, MD, PhD, 660 S Euclid, Box 8020, Washington University, St Louis, MO 63110.**

(NW5670)A

UNIVERSITY OF MANCHESTER

Department of Biochemistry and Molecular Biology

RESEARCH ASSOCIATE

Applications are invited for the appointment of a Postdoctoral Research Fellow to join a large research grouping funded by the SERC Animal Cell Biotechnology Programme. The research, under the supervision of Professor Keith Gull and Drs Alan Dickson and Martin Humphries, will examine the induction and enhancement of gene expression in recombinant mammalian cell lines and the role of serum growth factors and the extracellular matrix in the regulation of recombinant product formation.

The appointment tenable from October 1st, 1990 or as soon as possible thereafter is for a period of 2 years.

Initial salary will be within the range £11,399 — £18,165 p.a. according to the scale for Research Staff Grade 1A.

Experience in at least one of the following areas would be desirable: molecular biology, mammalian cell culture, protein purification, growth factors and their receptors.

Informal enquiries to Dr Alan Dickson (061 275 5077). Formal applications in writing, including a c.v. and the names and addresses of two academic referees, should be sent to Dr A.J. Dickson, Department of Biochemistry and Molecular Biology, the University, Manchester M13 9PT by September 3rd, 1990.

The University is an Equal Opportunity Employer. (4428)A

POSTDOCTORAL POSITION

Available immediately to study regulation of protein kinase C and/or sphingolipid biochemistry.

Submit curriculum vitae and three letters of reference to: **Dr Yusuf Hannun, Department of Medicine, Box 3355, Duke University Medical Center, Durham, NC 27710.**

An Affirmative Action/Equal Opportunity Employer.

(NW5666)A

UNIVERSITY OF WARWICK

SERC-DTI LINK

Programme in Biotransformations

Applications are invited from organic chemists and biochemists for a number of postdoctoral and postgraduate positions under this programme. Organic chemists should have experience in microbiological techniques and enzyme isolation and purification.

Salary on either the Research Range 1B scale: £11,399 — £13,495 pa or 1A: £11,399 — £18,165 pa.

Application forms from the Personnel Office, University of Warwick, Coventry CV4 7AL (0203) 523627 quoting Ref 2/2A/90/4 (please mark clearly on envelope).

Closing date for applications 13th September, 1990.

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nature

THE INTERNATIONAL JOURNAL OF SCIENCE
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Applications are invited for this post from persons with a background in the natural sciences who have:
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*respect within the research community
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The Deputy Editor will report directly to the Editor. The essential function of the post is to help to enhance the reputation and influence of *Nature* worldwide, but especially in the United States. Editorial input to some section of *Nature* will be an integral part of the job, as will be leadership and management of the Washington office.

Salary from $60,000.

Applications, marked CONFIDENTIAL, should include a full *curriculum vitae* (resume) together with supporting material and should be sent to the Editor, John Maddox (at either the London or Washington offices) no later than 7th September, 1990.

(4335)A

UNIVERSITY OF CAMBRIDGE
Department of Biochemistry

POSTDOCTORAL RESEARCH ASSOCIATES
in Biochemistry/Molecular Biology and NMR Spectroscopy

Applications are invited for two three year postdoctoral positions funded by the SERC to study the molecular recognition of DNA by eukaryotic gene regulatory proteins. The work will use biochemical/molecular biological and NMR spectroscopic techniques to study the structure and function of these proteins. Applicants with experience in either biochemistry/molecular biology or NMR spectroscopy would be very welcome, but candidates with other relevant backgrounds are encouraged to apply.

The posts are available from October 1990 at a starting salary within the range £11,399-£16,755 according to age and experience. Applicants should apply as soon as possible, sending a full CV naming two academic referees, to Dr E D Laue, Department of Biochemistry, University of Cambridge, Tennis Court Road, Cambridge CB2 1QW, UK (telephone Cambridge 333677/9) from whom further details may be obtained.

The University follows an equal opportunities policy. (4426)A

UCL

UNIVERSITY COLLEGE AND MIDDLESEX SCHOOL OF MEDICINE
DEPARTMENT OF PHYSIOLOGY

Post-Doctoral Research Assistant (Grade 1A)

A vacancy exists for a suitably qualified candidate in a new group investigating the annexin family of calcium binding proteins. The position is funded by a grant from the Medical Research Council for three years, and focuses on the identification of annexin homologues in lower eukaryotes, with the intention of studying their function within the context of the cell cycle.

Apply in writing, enclosing two copies of your c.v. to Dr Stephen E. Moss, at the Department of Physiology, University College London, Gower Street, London WC1E 6BT. The starting salary according to age and experience £16,000-£17,500, and the position is available immediately. Tel: 071-387 7050, ext. 3250.

Equal Opportunities Employer

DIRECTOR OF PROGRAM IN IMMUNOBIOLOGY
Northwestern University

Qualified candidates are sought for the new position of Director of the Immunobiology Program at Northwestern University Medical School. The Immunobiology Program is a newly developed interdepartmental effort designed to enhance research and training in immunobiology within Northwestern University as a whole with emphasis at the Medical School. With resources provided by both the Medical School and University Administrations, the new Director of Immunobiology will be expected to provide leadership in this effort, including the recruitment of new faculty.

Administrative duties will be minimal and compatable with continuation of an active research program.

To be considered for this position, a candidate must be recognized as a leading contributor in the area of immunobiology and must present plans for the further development of his/her own research program. The academic appointment at Northwestern University will most likely be in the Department of Microbiology-Immunology, although this is negotiable.

Rank and tenure status will depend upon the experience and background of the appointee. Initiation of the appointment could be at any time during the 1990/91 academic year. In order to ensure full consideration, applications consisting of *curriculum vitae*, summary of research interests, and the names of three references should be received by 15th October, 1990.

Applications should be addressed to Immunobiology Program Search Committee Chairman, Department of Microbiology-Immunology, Northwestern University Medical School, 303 East Chicago Avenue, Chicago, Il 60611.

Northwestern University is an Affirmative Action/Equal Opportunity Employer.

Hiring is contingent upon eligibility to work in the United States. (NW5675)A

CARLETON UNIVERSITY, OTTAWA, CANADA

MOLECULAR MICROBIOLOGY

Postdoctoral Fellowship in Molecular Microbiology to study regulation of plasmid replication, inheritance and host range — see J. Mol. Biol. **213**, 777 (1990); Gene **91**, 1 (1990); Plasmid **17**, 161 (1988). A recent Ph.D. with experience in molecular genetics and well-motivated to such studies would qualify. Three years. Stipend range: $22-25000 Can. **Write with a c.v., names and addresses of at least 2 persons who can provide confidential appraisals. Prof. V.N. Iyer, Dept. of Biology, Carleton University, Ottawa, On., Canada K1S 5B6.** (NW5679)A

PROTEIN CHEMISTS AND CELL BIOLOGISTS IN VASCULAR RESEARCH

Research positions (postdoctoral fellow to senior research scientists) immediately available for qualified MD's/PhD's to participate in multiple NIH-funded research projects related to interaction of complement and coagulation systems with platelets and vascular endothelium. Prior training or expertise in physical biochemistry, protein chemistry, molecular biology and/or cell biology desired.

Contact: **Peter J. Sims, MD, PhD,**
Oklahoma Medical Research Foundation,
825 N.E. 13th Street, Oklahoma City, OK 73104.

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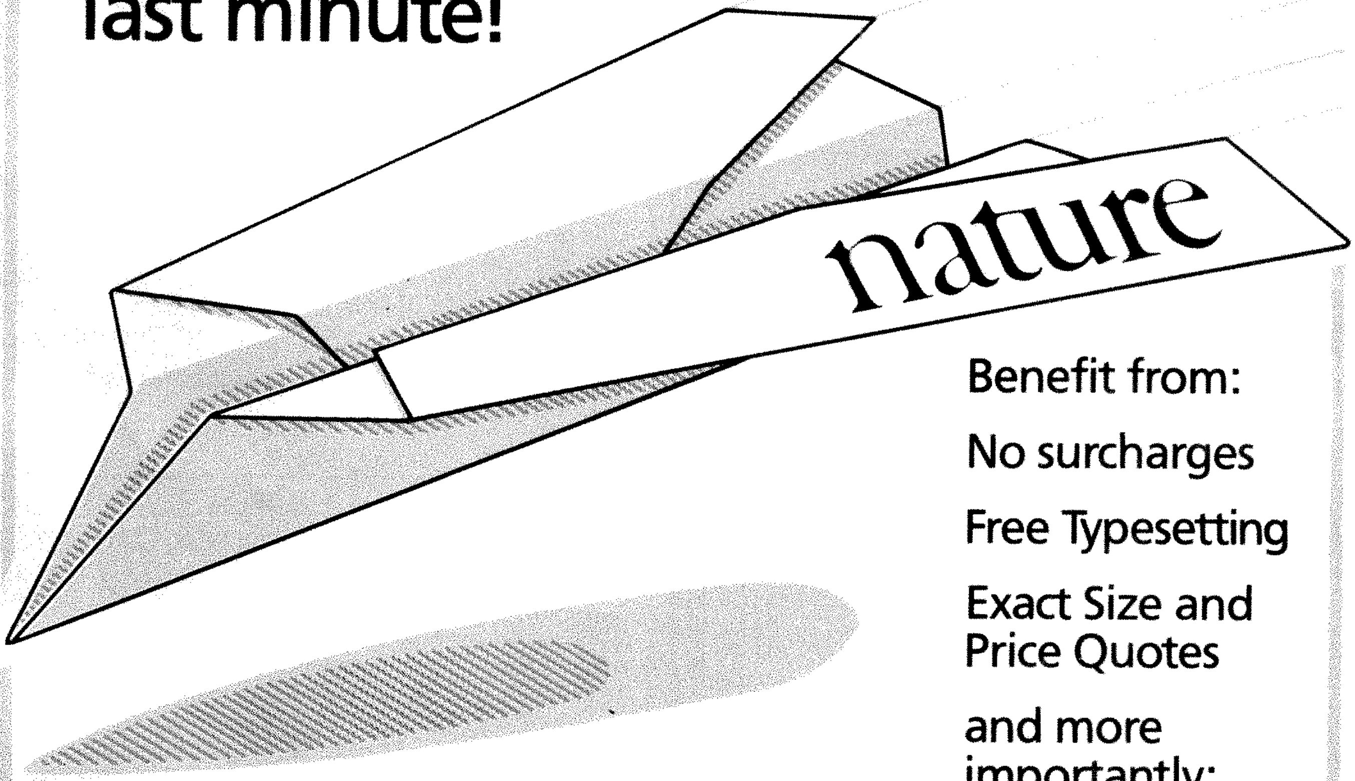

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NASA

The Goddard Space Flight Center
is seeking candidates for the position of
Head, Solar Physics Branch, Laboratory for Astronomy and Solar Physics

The Branch carries out a broad observational and theoretical solar research program using space and ground-based observations. Areas of interest include the deep solar interior and the solar dynamo, coronal heating and the solar wind, solar flares and other manifestations of solar activity. Current research programs are conducted in four major areas: UV/EUV observations from SMM, rockets and instruments on SoHO and OSL; hard X-ray and gamma-ray observations from SMM, balloons, and the Mars Observer; ground-based visible-flight magnetograph and helioseismology observations, and solar plasma physics theory and numerical modelling. The Branch Head is responsible for formulating and advocating a continuing vigorous research and development program in Solar Physics. He/she is also responsible for the supervision of Branch personnel and the management of Branch resources. Candidates should have professional experience equivalent to at least GM-14 in the Federal Service and be actively engaged in advanced research, and show demonstrated capability in leading and developing such research efforts. A PhD degree in physical sciences or engineering is desirable.

The salary range if $59,216 to $76,982 per annum (grade GM-15) depending on experience. Applicants should submit a resume or application for Federal Employment (SF-171) by September 30th, 1990 to NASA/Goddard Space Flight Center, Employment and Employee Services Branch, Code 115SOA, Greenbelt, MD 20771. Technical inquiries should be directed to Dr Michael Hauser at (301) 286-8701.

Equal Opportunity Employer. US Citizenship required. (NW5650)A

NATIONAL HEART AND LUNG INSTITUTE
(ROYAL BROMPTON HOSPITAL)
ROYAL POSTGRADUATE MEDICAL SCHOOL
(HAMMERSMITH HOSPITAL)
(University of London)

RESEARCH ASSISTANT AND TECHNICIAN

Applications are sought for two posts to work on a British Heart Foundation funded project to study the innervation of the conduction system in the human heart, using modern morphological techniques. The work is part of an ongoing collaboration between the two centres which offer excellent research facilities and an exciting working environment.

Research Assistant

Preference will be given to a candidate with a good honours degree and relevant experience, but full practical training will be given. The candidate will be expected to register for a higher degree (MPhil/PhD) and must be able to work independently.

Research Technician

The candidate will have a key role in the project and should have a degree or training in pure or applied biological sciences. Practical experience in histology and immunohistochemical techniques would be an advantage, but it not essential, further training for higher degree possible.

Both posts are available from 1st October, 1990 and will be in the salary range £7,897 — £12,086 plus London Allowance. Informal enquiries may be made to Dr Mary Sheppard (071 352 8121, ext. 4942) or Prof. Julia Polak (081 740 3231).

To apply please send a CV with the names and addresses of 2 referees to the Personnel Manager, National Heart and Lung Institute, Dovehouse Street, London SW3 6LY quoting ref no AXB1/125 by the 7th September, 1990. (4445)A

FACULTY POSITION
MARINE SCIENCE

The Institute of Marine Science, School of Fisheries and Ocean Sciences, is seeking an outstanding scientist for a tenure track position in teaching and research in the area of oceanography with interest in remote sensing techniques. The position will be filled at the Assistant Professor level or above depending on background and experience. The successful applicant will be expected to develop a vigorous research program in oceanography oriented toward remote sensing of high latitude waters, as well as participate in teaching and guidance of graduate students. Examples of potential areas of research include primary production, environmental changes associated with global warming, or marine mammal migrations. The University of Alaska Fairbanks is developing a major remote sensing capability through the Alaska SAR facility that will offer new opportunities in this area of research. The Institute of Marine Science has a faculty of 26 with a graduate enrollment of 40-50 students. The faculty are involved in a variety of research programs emphasizing arctic and high latitude seas.

Applicants should send a summary of research interests with a curriculum vitae and the names, addresses, and phone numbers of three references to:

Donald M. Schell,
Chair, Remote Sensing Search Committee,
c/o Director's Office,
Institute of Marine Science,
University of Alaska Fairbanks,
Fairbanks, AK 99775-1080.

Closing date for applications is 15th October, 1990. Applications of finalists may be subject to public disclosure. Persons hired must comply with the new employment provisions of the Federal Immigration Reporting Act. The University of Alaska is an Equal Opportunity/Affirmative Action Employer.

(NW5646)A

DISCOVER THE NEW ARABIA

The King Faisal Specialist Hospital and Research Centre in Riyadh, Saudi Arabia has the following positions available:

RESEARCH FELLOW

Applicants should have a PhD and a minimum of 3 years relevant experience in molecular biology and/or pharmacogenetics. Must be capable of handling various biochemical methods. Research would include application of molecular biology in medicine.

RESEARCH FELLOW

Applicants should have a PhD and a minimum of 3 years relevant experience in molecular biology and/or molecular genetics. Must be capable of handling various biochemical methods. Research would include application of molecular biology in medicine.

SENIOR RESEARCH TECHNICIAN

Must be capable of handling various biochemical methods. Experience in molecular biology and/or pharmacogenetics is desirable. Minimum of 7 years research experience with BS degree in appropriate science required. (Some reduction of experience possible with MS).

Benefits include potentially tax-free salary, liberal vacation and holidays, furnished housing and air transportation. To apply, send resume to:

HOSPITAL CORPORATION INTERNATIONAL
2515 Park Plaza
Nashville, TN 37203

(NW5684)A

UNIVERSITY OF GLASGOW

LECTURESHIP IN VETERINARY ANIMAL HUSBANDRY

Candidates are invited to apply for a lecturship in Veterinary Animal Husbandry which will be for a two year period in the first instance commencing 1st January, 1991. The appointment principally involves undergraduate teaching of veterinary students together with contributory roles in the research and administration of the department. Applicants should preferably hold a veterinary degree and have postgraduate experience with farm animals, small animals and horses. An established research expertise in an appropriate area would be to advantage.

The department is responsible for the instruction of first and third year veterinary students in the management, welfare, nutrition, housing and breeding of most animal species and the teaching involves a large practical element. The department also has a major role in the management of the University farm at Cochno.

Salary will be within the Lecturer scale (£12,086 – £22.311) with placement according to age, qualifications and experience.

Further particulars may be obtained from the Academic Personnel Office, University of Glasgow, Glasgow G12 8QQ, where applications (8 copies), giving the names and addresses of three referees should be lodged on or before 7th September, 1990.

In reply please quote ref. no. 7061. (4448)A

THE UNIVERSITY OF SHEFFIELD

INSTITUTE OF ENDOCRINOLOGY

Department of Human Metabolism and Clinical Biochemistry

POST-DOCTORAL RESEARCH ASSISTANT

Applications are invited for the above post tenable for one year in the first instance. The successful applicant will join a team investigating signal transduction mechanisms in a variety of cell systems. Programmes currently under way include studies on defects in cellular signalling in cystic fibrosis, cytokine and hormone action in lymphoid tissue and interactions between signalling systems.

Applicants should possess a PhD in Biochemistry or other relevant discipline. The salary will be on Range 1A (£11,399-£18,165), according to age and experience.

Applications, including a full curriculum vitae and the names and addresses of two academic referees, should be sent to Professor B L Brown, Institute of Endocrinology, Department of Human Metabolism and Clinical Biochemistry, The University of Sheffield Medical School, Beech Hill Road, Sheffield, S10 2RX, by 7th September, 1990. Reference MAP311/G.

AN EQUAL OPPORTUNITY EMPLOYER (4438)A

UNIVERSITY OF LIVERPOOL

Department of Physiology

TWO POSTDOCTORAL SENIOR RESEARCH ASSISTANTS

Financed by the The British Heart Foundation (BHF) and the MRC. The work is centred around smooth muscle metabolism and function.

The BHF post is for up to two years to investigate the relation between pH and vascular tone, at the cellular level, using pH and calcium sensitive indicators. pH is an important physiological and pathological determinant of tone but the mechanism is unclear.

The MRC post is for up to three years to investigate uterine pH — its regulations and effects upon contraction. There is evidence that uterine pH changes during labour and may influence its outcome. Isolated cell and uterine strips will be used to measure pH and contraction and investigate this area.

The Department of Physiology has a UFC grade 5 rating and provides excellent support for research in a friendly atmosphere.

Informal enquiries to Dr. S. Wray (051 794 5306).

Initial salaries within the range £12,086 — £14,744 per annum.

Applications, by CV with the names of three referees, should be received as soon as possible by the Director of Staffing Services (AS), The University, P.O. Box 147, Liverpool L69 3BX, from whom further particulars may be obtained. Quote ref. RV/796.

An Equal Opportunity Employer. (4449)A

VIROLOGIST

The Yerkes Regional Primate Research Center and Department of Pathology, Emory University have an opening for a virologist with experience in retrovirus research. Candidates should have a PhD, DVM, MD, or equivalent and should have sufficient research experience to demonstrate their ability to establish an independent research program in areas related to the pathogenesis of AIDS and related retroviruses in animal models. The successful applicant will also participate in ongoing clinical, immunological and pathological studies with the simian immunodeficiency virus.

Salary is negotiable and is commensurate with experience. The position is available immediately.

Letters of application including a curriculum vitae, a brief statement of research interests and the names of three references should be sent to **Dr Harold McClure, Associate Director, Yerkes Primate Research Center, Emory University, Atlanta, GA 30322.**

Emory University is an equal opportunity affirmative action employer. (NW5656)A

ASSISTANT RESEARCH SCIENTIST

Recent PhD with training in molecular biology (cloning; PCR; Northern, Southern) and immunology. Project: Gene expression in human T-cells and Thymocytes; effect of immunosupressant drugs. Excellent benefits include Medical/Dental/Prescription and NYU Tuition.

Send resume to: Joan Harmon, Personnel Department, NYU Medical Center, 550 First Avenue, Kips Bay Room C-47, New York, NY 10016.

An Equal Opportunity Employer M/F. (NW5682)A

POSTDOCTORAL FELLOW/RESEARCH ASSOCIATE

Available immediately to study proteases in mammalian cells and their role in cancer and muscle and heart disease. Salary: $24,000 per year for 2 to 5 years.

Send curriculum vitae to: **Drs Julie Fagan and Lloyd Waxman, Animal Science Department, Rutgers University, PO Box 231, New Brunswick, NJ 08903. Tel: 201-932-8354. Fax: 201-932-6996.** (NW5686)A

RESEARCH FELLOW

Massachusetts General Hospital/Harvard Medical School.

Immediate opening for PhD in Organic Chemistry as Research Fellow. Research project involving development of synthesis and labelling methods or short lived radiopharmaceuticals. Excellent academic environment coupled to pleasant Boston living. Address correspondence to: **Dr David Elmaleh, Massachusetts General Hospital, Radiology, Boston, MA 02114 or call 617-726-3805; Fax 617-726-5123.** (NW5681)A

UNIVERSITY OF MANCHESTER

School of Biological Sciences

LECTURESHIP IN MOLECULAR BIOLOGY

Applications are invited for a new Lectureship in Molecular Biology to be held in the Department of Biochemistry and Molecular Biology. Applications are invited from candidates using molecular biological approaches to the study of eukaryotic cells or organisms.

The appointment will be made on the Lecturer scale A (£12,086 – £16,755 p.a.) or B (£17,455 – £22,311 p.a.) according to qualifications and experience.

Further particulars may be obtained from the Registrar, The University, Manchester M13 9PT (tel: 061 275 2028, quote ref. 246/90, with whom applications (containing a curriculum vitae and the names of three academic referees) should be lodged no later than October 1st, 1990. Informal enquiries regarding the post may be made to Professor Keith Gull (061 275 5108).

The University is an Equal Opportunity Employer. (4427)A

RESEARCH ASSOCIATE

Operate an ES Cell Transgenic Mouse Facility. The University of Cincinnati College of Medicine is establishing a service facility that will produce germline chimeric mice from ES cells. Applicant must have experience in blastocyst injections, ES cell culture, mouse embryo manipulation and maintenance of a mouse breeding colony. $30,000 salary and good benefits package. Send curriculum vitae and references to Tom Doetschman, University of Cincinnati College of Medicine, Department of Molecular Genetics, Biochemistry and Microbiology, 231 Bethesda Ave, (ML 524), Cincinnati, Ohio 45267-0524.

The University of Cincinnati is an Equal Opportunity / Affirmative Action Employer. (NW5677)A

EMORY UNIVERSITY SCHOOL OF MEDICINE

Department of Microbiology and Immunology

CHAIR

Emory University School of Medicine invites nominations and applications for the position of Chair for the Department of Microbiology and Immunology. Responsibilities include the direction, management, and continuing development of a department committed to excellence in research and teaching in a university setting. The faculty has teaching responsibilities for the MD degree program of the Medical School and also for the PhD degree programs offered through the Graduate School of Arts and Sciences. The department is located in ultramodern facilities in the Rollins Research Center on the Emory Campus and is adjacent to the Centers for Disease Control. The department has a very active research effort and candidates must hold an MD or PhD degree with training in mocrobiology or immunology, and have a strong record in research. Previous academic and administrative responsibilities are required, as well as demonstrated leadership capabilities. Salary is negotiable.

Applications should include a current curriculum vitae and the names of appropriate references, and should be submitted no later than October 1st, 1990 to:

Microbiology and Immunology Search Committee
312 Woodruff Health Science Center
Emory University School of Medicine

Emory University is an Equal Opportunity/Affirmative Action Employer. (NW5683)A

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(4450)A

UNIVERSITY OF GLASGOW

Department of Zoology

POSTDOCTORAL RESEARCH ASSISTANT

Applications are invited for the above post for 3 years from September 1990 or soon after, in the Zoology Section of the Wellcome Laboratories for Experimental Parasitology. The position is funded by the Wellcome Trust to work on the molecular biology/biochemistry of antigenic variation in rodent malaria parasites. A graduate research assistant will also be employed on the project. Applicants should have experience in biochemistry and/or molecular biology, be capable of independent research, and desirably have some experience in parasitology or microbiology.

Starting salary will be up to £13,527 depending on age and experience, with annual increments thereafter.

Applications including a curriculum vitae and the names of at least two referees should be sent to Professor R S Phillips, Department of Zoology, University of Glasgow, Glasgow G12 8QQ, to whom informal enquiries can be made on 041-339 8855 ext 4776.

(4429)A

UNIVERSITY OF BRISTOL

LECTURER IN DERMATOLOGY/APPLIED IMMUNOLOGY

JPB/Vet/6

Applications are invited for a Lectureship in Dermatology/Applied Immunology within the Department of Veterinary Medicine at Bristol University. The appointed person will take particular responsibility for dermatology, especially in the inter-relationship with applied immunology, although applications will also be considered from candidates with an interest in immunoparisitology. The successful candidate will be expected to initiate research in an appropriate field. The current salary scale is £12,086 to £22,311 depending on age and experience.

Persons interested in discussing the post prior to application should contact Professor Philip Duffus, Department of Veterinary Medicine, Langford House, Langford, Bristol BS18 7DU (0934 852581).

Applications: These should take the form of 2 copies of a curriculum vitae, include the names and addresses of 3 referees and be received by the Registrar, University of Bristol, Senate House, Tyndall Avenue, Bristol BS8 1TH, by 14th September 1990. Please quote reference JPB/Vet/6. (4432)A

THREE POSTDOCTORAL POSITIONS

to study 1) molecular biology of skin cytochrome P-450 and skin cancer, 2) skin photosensitization and photodynamic therapy of skin cancer, 3) diet and cancer chemoprevention. PhD with relevant experience desirable.

Send CV with names of refs to: Professor Hasan Mukhtar, Research Director, Department of Dermatology, Case Western Reserve University, Cleveland, Ohio 44106.

(NW5668)A

POSTDOCTORAL POSITION

MOLECULAR NEUROBIOLOGY

Applicants invited to study the molecular basis of regulation of catacholamine biosynthetic ensymes.

A PhD in biochemistry, cell or molecular biology is required and experience in recombinant DNA research and neuroscience is desirable.

Competitive salary and benefits. Attractive setting in Westchester County about 15 miles north of New York City.

Contact: Dr. Esther L. Sabban, Department of Biochemistry and Molecular Biology, New York Medical College, Valhalla, N.Y. 10595 U.S.A. Phone: (914) 993-4069 or (914) 993-4055.

(NW5678)A

UNIVERSITY COLLEGE LONDON

Department of Physiology

UCL

POSTDOCTORAL RESEARCH ASSISTANT

to work with Dr. Stephen Bolsover on the role of calcium and other intracellular messengers at the nerve cell growth cone (see Nature *343*, p741). Techniques employed include whole cell and single channel patch clamp and digital fluorescence imaging. The post is tenable for three years. Starting salary up to £19,222 (including London Weighting) depending on age and experience.

Applications, with a full curriculum vitae and names of at least two referees, should be sent to Dr. Stephen Bolsover, Department of Physiology, University College London, Gower Street, London WC1E 6BT, from whom further particulars can be obtained (Tel. 071 387 7050, ext. 3269 or 3257).

The University follows and Equal Opportunity Policy. (4444)A

FELLOWSHIPS

UNIVERSITY OF KENT AT CANTERBURY

Physics Laboratory

FIVE RESEARCH FELLOWSHIPS

Applications are invited for five posts from Postdoctoral Physicists or Electronic Engineers with experience in modern optics (or graduates with relevant industrial experience) to undertake research into optical fibre based sensors and high bandwidth optical signal processing.

Post 1: Current and voltage sensors based upon interactions such as the Faraday effect for monitoring of electrical machines and power distribution (Ref A91/2).

Post 2: Development of miniature triple axis accelerometers with sub 'micro g' resolution for seismic surveying applications, for example bore hole vertical seismic profiling (Ref A91/3).

Post 3: Development of optical communications and ranging systems based upon coherent optical correlation techniques (Ref A91/4).

Post 4: Research into novel forms of distributed sensors funded by SERC via the recently formed Optical Research Centre (ORC) at Southampton (Ref A91/5).

The above posts are for three years. Salaries on the RA1A scale (£11,399 — £18,165 p.a.).

Post 5: The completion of an optical system capable of measuring total velocity field in gas flow. This post is funded by Shell and for one year initially. Salary will be on the RA1A Scale (Ref A91/6).

Informal enquiries to Professor D.A. Jackson (0227 764000, ext. 3764). Application forms and further particulars may be obtained from **Mr R.T. Shine, The Registry, The University of Kent at Canterbury, Canterbury, Kent CT2 2NZ**, quoting the appropriate reference number. The closing date for applications is 7th September 1990.

An Equal Opportunities Employer. (4435)E

POSTDOCTORAL FELLOWSHIP

Hematology Unit and a new and rapidly growing Bone Marrow Transplant Division looking for PhD to spearhead effort studying the molecular biology of hematopoietic growth factors and their receptors. Interactions of growth factors with stem cells, pathways of signal transduction, growth factor-induced signal transduction and gene expression will be studied. Interactions with other members of the group interested in stem cell biology, embryonic stem cells, and the hematopoietic microenvironment encouraged. Interested applicants with significant molecular biology experience preferred. Applicants should submit curriculum vitae with names of references to **John F. DiPersio, MD, PhD, University of Rochester Medical Center, Hematology Unit, 601 Elmwood Ave., PO Box 610, Rochester, NY 14642.** *EOE M/F.* (NW5658)E

POST DOCTORAL FELLOWSHIP

THE JOSE CARRERAS INTERNATIONAL FOUNDATION FOR THE FIGHT AGAINST LEUKEMIA

Funding is for research in the field of leukemia or related hematologic disorders. The award provides $50,000 per year, $35,000 of which may be used for salary. The award may be renewed for an additional two years. Deadline for applications is January 1, 1991.

One applicant per institution. For forms and details write:

From the Eastern Hemisphere
Alberto Granena, M.D., Postgraduate School of Hematology, Villarroel, 170, 08036 Barcelona, SPAIN

From the Western Hemisphere
E. Donnall Thomas, M.D., Hutchinson Cancer Center, 1124 Columbia Street, Seattle, WA 98104.

(NW5655)E

UNIVERSITY OF YORK

MRX AIDS Directed Programme

DEPARTMENT OF CHEMISTRY

POSTDOCTORAL RESEARCH FELLOWSHIP

Required for a postdoctoral research position suited to a molecular biologist with an interest in protein structure and function.

You will be involved in a programme of mutagenesis and expression of truncated forms of the HIV envelope protein, gp120. The aim of this project is to investigate the structural basis of HIV binding to its receptor, CD4. This is a joint collaborative project between the University of York, NERC Institute of Virology, Oxford, and Celltech Limited, and is funded by the MRC AIDS Directed Programme, You should have experience in gene manipulation and, preferably, in expression of recombinant proteins in either mammalian or insect cells.

Salary within the range: £11,399-£14,744 per annum. The appointment is available for three years.

Three copies of applications with full curriculum vitae and the names of two referees, should be sent by Friday 14 September 1990 to the Personnel Office, University of York, Heslington, York YO1 5DD; please quote reference number 6/6322.

GRADUATE STUDENTSHIP

Also required for a three-year research studentship in protein crystallography. You will join a challenging project of crystallographic studies of the HIV receptor, CD4. You should hold at least an upper second class honours degree in a suitable discpline.

Initial enquiries for both positions should be directed to Dr L Brady, Department of Chemistry (tel: 0904 432572), from whom further particulars are available.

(4461)E

continued on page 2

Laboratoire de Physiologie et Biologie Moléculaire Végétale

Université de Perpignan — France

POST-DOCTORAL FELLOWSHIP

Applications are invited for a post-doctoral EEC BRIDGE fellowship tenable for 2 years plus one year renewable. The project involves the use of T-DNA tagging in *Arabidopsis thaliana* to isolate and identify novel genes expressed during embryogenesis. A background in tissue culture and/or molecular biology is desirable. Applicants of other plant biology fields will be considered. The project is run in collaboration with Leicester University (UK) and Danisko (DK). French speaking is not necessary.

Starting date is 1st January, 1991.

Applicants should send a *curriculum vitae* and the names and addresses of two referees to: Dr P Gallois/Dr M Delseny, Laboratoire de Physiologie et Biologie Moléculaire Vegetable, Université de Perpignan, 52 Av de Villeneuve 66025 Perpignan CEDEX—FRANCE, from whom further information can be obtained (Tel: +33 68 66 88 48. Fax: +33 68 66 84 99). (W7668)E

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STUDENTSHIPS/AWARDS

SIR WILLIAM DUNN SCHOOL OF PATHOLOGY

RESEARCH STUDENTSHIP IN MOLECULAR VIROLOGY

A research studentship is available to study the molecular biology and enzymology of African Swine Fever Virus (ASFV). ASFV has many similarities with poxviruses and causes acute haemorrhagic fever in swine that poses an increasing economic threat to British agriculture. The project will involve the mapping and sequencing of the ASFV DNA ligase gene and biochemical characterization of the enzyme activity. The project is funded by an AFRC studentship and will be jointly supervised by Dr Geoffrey L. Smith in Oxford and Dr Linda Dixon in The Institute for Animal Health, Pirbright.

The applicant will be based in Oxford for the gene mapping, sequencing and expression studies, but all work with infectious virus will be performed under containment conditions in Pirbright where assistance with temporary housing may be available.

The studentship is available to EEC nationals only.

Applicants should send their CV and the names and addresses of two referees to The Administrator, Sir William Dunn School of Pathology, Oxford University, South Parks Road, Oxford OX1 3RE.

Informal enquiries may be made to Dr G.L. Smith (0865) 275521 or Dr L. Dixon (0483) 232441.

Closing date for applications 21st September, 1990. (4455)F

ST GEORGE'S HOSPITAL MEDICAL SCHOOL
(University of London)

PhD STUDENTSHIP

Applications are invited for a studentship beginning in October, 1990 rom candidates who hold at least an upper second class honours degree on pharmacology or physiology. Applicants must be British citizens. Research is concentrated in the areas of smooth muscle and neuroscience and extends from the most fundamental basic research to linical investigation. The present position is to study the mechanism of action of vasoactive substances with electrophysiological and biochemical techniques. It is presently training a number of students for the PhD degree.

nterested candidates should contact Dr W A Large, Department of Pharmacology, St George's Hospital Medical School, Cranmer Terrace, London SW17 0RE (Tel: 081 672 9944, ext. 55617), enclosing their curriculum vitae and names of two referees. (4466)F

UNIVERSITY OF READING
School of Plant Sciences

SCHOOL STUDENTSHIP

Applications are invited for a University funded studentship to work in one of the following fields of research.

1. The role of Ca^{2+} regulated proteins in environmental sensing.
2. *In vitro* studies on heat stress and starch production in potato.
3. A revision of the genus *Quercus* in the Mediterranean region.

The conditions of employment and salary will be according to Research Council Guidelines, and the student will also be expected to demonstrate to practical classes.

Applications, together with a CV and the names and addresses of two referees should be sent without delay to **Professor Hugh Dickinson, School of Plant Sciences, Plant Science Laboratories, University of Reading, Whiteknights, Reading RG6 2AS,** from whom further details may be obtained.

Closing date: September 14th, 1990. (4421)F

THE MICHELSON-MORLEY AWARD

The prestigious Michelson-Morley Award is presented by Case Institute of Technology to recognize the distinctive achievements of a particular scientist or engineer. The award is named in honor of Albert A Michelson, first professor of physics at Case Institute and 1907 Nobel laureate, and Edward W Morley, distinguished professor of chemistry at Western Reserve College. Joint research by Professors Michelson and Morley is highlighted by their experiments related to the velocity of light. These scientific probes prepared the way for the discoveries of Albert Einstein and decisively advanced modern theoretical physics.

The stated purpose of the award is to honor the individual chosen for eminent contributions to knowledge and to the well-being of mankind, to perpetuate the tradition of scholarly inquiry exemplified by the independent and collaborative research of Professors Michelson and Morley, and to call to the attention of the general public the continuing contributions of the scientist and engineer to human welfare.

Deadline for nominations: November 15th, 1990.

Nominations should be sent to: Glenn R Brown, Dean of the Colleges, Case Western Reserve University, Crawford Hall 7100, 10900 Euclid Avenue, Cleveland, OH 44106. (NW5669)N

THE ROCKEFELLER FOUNDATION

African Dissertation Internship Awards

Doctoral students from sub-Saharan Africa are invited to apply to The Rockefeller Foundation for dissertation research support. The program enables PhD students enrolled in US and Canadian universities to return to Africa for extensive field research in areas relevant to economic development or poverty alleviation. Priority is given to research topics in the fields of agriculture, health and life sciences, but other proposals are welcome.

Applicants are responsible for arranging affiliation with an African institution able to provide needed research support, such as laboratory facilities, access to study sites, and technical advice. The candidate's faculty advisor, the host institution in Africa, and the agency with primary responsibility for financing the student's graduate work must all send letters of endorsement.

Deadlines for applications are October 1st, 1990 and March 1st, 1991. Candidates should apply well in advance of the expected field work starting date.

For a full description of the competition and the application requirements, write to: African Dissertation Internship Awards, The Rockefeller Foundation, 1133 Avenue of the Americas, New York, NY 10036 USA.

(NW5644)N

UNIVERSITY OF DURHAM
Department of Biological Sciences

Research Studentship in Cancer Research

A research studentship, funded by the North of England Cancer Research Campaign, to investigate the effect of heat on disruption of intracellular signals (especially inositol phosphates, calcium and 1.2-diacylglycerol) in relation to hyperthermic death of tumour cells. Applicants should have a good honours degree in biological science, together with a strong interest in animal biochemistry. Further information can be obtained from Mrs J. Mather (Departmental Secretary). Tel: 091-374 3342.

Applications, including a c.v. and names of two referees, should be submitted by 7th September, 1990, to Dr R. Manning, Dept. of Biological Sciences, University of Durham, South Road, Durham DH1 3LE. (4361)F

FELLOWSHIPS continued

UNIVERSITY OF EDINBURGH
MRC AIDS DIRECTED PROGRAMME
RESEARCH FELLOW

A Post-Doctoral Research Fellowship is available in a substantial research group located in the Department of Genetics, King's Buildings, Edinburgh, investigating sequence variation in HIV and its immunological significance in patients.

The position is grant supported until September 1993 and the salary will be at the appropriate point on the Range 1A scale. (£12,086-£18,165 with placing according to experience).

Candidates with either clinical or non clinical backgrounds in any aspect of biomedical science will be considered: a background in virology or immunology would be especially valuable.

For further information contact: Dr Andrew Leigh Brown in the Department of Genetics or telephone 031-667 1081, ext. 3551.

Applications should include the names and addresses of 3 referees and should be addressed to: The Personnel Office, University of Edinburgh, 63 South Bridge, Edinburgh.

Please quote ref: 5798. (4352)E

ASSISTANTSHIPS

UNIVERSITY OF EDINBURGH

Applications are invited for a

POST-DOCTURAL/PRE-DOCTORAL RESEARCH ASSISTANTSHIP

to work on the immunology of Plasmodium falciparum malaria in man.

The project will be based in the Centre for parasite Biology in Edinburgh (King's Buildings) with opportunities for field studies in west Africa and elsewhere.

Previous experience in cellular immunology would be an advantage.

The post is funded by the Wellcome trust for an initial period of three years from 1st October 1990.

Salary wil be at the appropriate point on the 1B (£12,086-£13,495) or the 1A (£12,086-£18,165) scale with placing according to qualifications and experience.

Informal enquiries can be made to Dr Eleanor Riley or Dr Richard Cater on 031-667 1081 ext. 3699 or 031-668 3861.

Applications, with curriculum vitae and the names and addresses of 3 referees, should be made to: The Personnel Office, University of Edinburgh, 63 South Bridge, Edinburgh, and should be received no later than 10th September, 1990.

Please quote reference 5797. (4351)P

SEMINARS & SYMPOSIA

BIOCATALYSIS FOR THE 90s

An International Conference on the New Science and Techniques for Discovery, Design, and Use of Biocatalysts

JUNE 5-7, 1991
Orlando (Lake Buena Vista)
Florida, USA
Program Chairman:
Sheldon W. May, PhD
Georgia Institute of Technology

The scientific program will consist of keynote lectures by invited speakers, contributed papers, and poster presentations reflecting the renewed emphasis on biocatalysis as biotechnology enters its third decade.

Contact: Rita S. Kessel, Butterworth-Heinemann,
80 Montvale Avenue, Stoneham, MA 02180 USA.
Telephone: (617)438-8464, ext. 293. FAX: (617)279-4851. (NW5661)M

continued on page 22

COURSES

ROYAL SOCIETY MEETINGS

THURSDAY 20 AND FRIDAY 21 SEPTEMBER: Discussio Meeting on **'BONDING AND STRUCTURE OF SOLIDS** organized by R. Haydock, J.E. Inglesfield and J.B. Pendry F.R.S.

Speakers: V. Heine, N.W. Ashcroft, J.D.C. McConnell D.G. Pettifor, J.C. Phillips, T.M. Rice, P.W. Anderson L.J. Sham, J.C. Inkson, M.L. Cohen, B.L. Gyorffy J.E. Inglesfield, J.B. Pendry and R. Haydock.

MONDAY 1 AND TUESDAY 2 OCTOBER: Discussio Meeting on **'DEVELOPMENT AND REGENERATION O THE NERVOUS SYSTEM'** organized by J.G. Nicholls F.R.S. and A. Aguayo.

Speakers: Y.-A. Barde, M.C. Raff, A. Davies, J. White B.G. Wallace, A. Lumsden, J.P. Brockes, P. Rakic E. Shooter, M.E. Schwab, C. Sotelo, K.J. Muller J.G. Nicholls and A.J. Aguayo. (4460)C

Registration details (no fee for attendance): Scientifi Meetings Secretary, The Royal Society, 6 Carlton Hous Terrace, London SW1Y 5AG (Tel: 071-839 5561, ext. 278.)

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(4450)A

ACTION THEMATIQUE ET INCITATIVE SUR PROGRAMME ET EQUIPE (ATIPE)

CALL FOR OFFERS: PROTEIN ENGINEERING

Application deadline: October 15th, 1990

The aim of the present ATIPE is to promote new groups in the area of protein engineering. Its goals are the following:

1 Training of investigations in the cited scientific fields and their recruitment at the end of the training program.

2 Integration of non statutary and statutary investigators in already established laboratories. In order to achieve the selected programs, they will receive technical help and financial support.

This action will be supported for a period of 3 years, by the Life Sciences, the Chemistry, the Mathematics and Basic Physics, and the Engineering Physics CNRS departments, the IMABIO program and the CEA Mission for Life Sciences.

Scientifics topics of program:

— Determination of the tridimensional structure of proteins by crystallography and crystallogenesis (including subsequent validation of the effects of microgravity).
— Determination of protein structures by high-resolution RMN.
— High-resolution electronic microscopy on bidimensional crystals.
— Modeling, molecular graphic representation, molecular dynamics and structural prediction, in correlation with an experimental approach.
— Protein engineering, structure-function relationships, alteration and generation of proteins with new properties. Investigation in new molecular biology methodologies, appropriate to these topics.

Modalities of application:

1 — Training program

Applicants should be post-doctoral trainees and/or investigators in data-processing, physico-chemistry and biology, willing to specialize in the research areas supported by the present program.

Applicants should propose a training schedule that will be supported by the ATIPE if selected. At the end of the training program, applicants will be considered for employment in a CNRS or a CEA research laboratory.

2 — Establishment and development of research groups

Directors of laboratories or of groups of laboratories should present a project for the creation of a new research group in one of the mentioned topics. They should show how this could help them to gain new insights in the domain of protein engineering. They will have to describe in term of surface, technical help and financial support, the means given to the new scientific action, as well as those expected from the ATIPE or from any other agency.

The scientific Committee will coordinate the proposals coming from individuals with those emanating from laboratories. Applications will be written in French or English and sent in the form of a declaration of intent with a short description of the scientific background and proposed scientific program.

Standard application forms will be available at CNRS: Mrs Ariel Gille (Phone: 33. 1.47.53.10.40) and the applications will be addressed to Mrs Ariel Gille, CNRS — 15 quai Anatole France, 75700 Paris, France.

DEPARTEMENT DES SCIENCES DE LA VIE DU CNRS
15 Quai Anatole France — 75700 PARIS
Tel: (33) 1. 47.53.15.15 — Telex: 260 034

(W7670)X

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Agency for International Development

Announcement of

Malaria Vaccine Research & Development Request for Applications [RFA-ST/H-90-001]

Background. For more than 20 years, the United States Agency for International Development (A.I.D.) has supported a program of applied research in malaria vaccine development. Currently, the primary research foci of the Malaria Vaccine Research & Development Project include:

—the identification and characterization of malaria parasite antigens for development of experimental vaccines;

—the elucidation of the importance of identified antigens and B-cell and T-cell epitopes in providing a protective immune response in naturally acquired human malaria and in humans and relevant animal models, including appraisals of strain specificity of protective immunity;

—the elucidation of humoral and cellular effector mechanisms of protective immunity to malaria, especially protection from the pathological consequences of infection, and the development of *in vitro* correlates of protective immunity in humans;

—the modification of the structure and/or mode of presentation of antigens which have been shown to have a role in protection, in an attempt to increase their ability to induce a protective immune response.

Proposals. A.I.D. is soliciting proposals for Cooperative Agreements describing a program for malaria vaccine research. In general, the range of activities requested in the RFA is as outlined above. However, there is a special interest in proposals whose major emphasis is on development of vaccines that induce responses mimicking natural immunity (*i.e.*, a state characterized by protection from disease but not necessarily from infection) thus allowing restimulation of immunity through natural exposure to parasites. It is the intent of A.I.D. to support 3-year research programs. Only U.S. Institutions are eligible for these awards, but collaboration with scientists in malaria endemic areas is encouraged.

Applications. Proposals must be received by November 2, 1990. Forms and detailed instructions as well as programmatic information are available from:

Project Officer
Malaria Vaccine Research & Development Project
A.I.D. Office of Health (SA-18; Room 705)
Washington, DC 20523
fax: 703-875-5490

(NW5643)X

COURSES/SEMINARS & SYMPOSIA continued

Frontiers in Basic Sciences That Relate to
Heart, Lung, and Blood Diseases Symposium:

Cell-Cell Interactions

November 28-29, 1990
Masur Auditorium
National Institutes of Health
Bethesda, Maryland

Sponsored by The
National Heart, Lung, and
Blood Institute (NHLBI) and
Fondazione Giovanni Lorenzini

The symposium is the fifteenth in the series of "Frontiers in Basic Sciences That Relate to Heart, Lung, and Blood Diseases." This series is conducted by NHLBI to capitalize on and transfer the progress achieved in basic science disciplines to clinical research programs. Through the pursuit of new knowledge concerning the fundamental aspects of the life processes relating to heart, lung, and blood diseases, it is hoped that better approaches to disease prevention and control will be discovered. At this symposium, leading researchers and experts in the field will present their views on the state of the science, the problems facing current understanding, and anticipated future developments in the research of cell-cell interactions.

The symposium will be cochaired by Dr. K. Frank Austen of Brigham & Women's Hospital, Boston, Massachusetts, and Dr. Rodolfo Paoletti of the Fondazione Giovanni Lorenzini, Milan, Italy. Presentations will be structured around the following topics: leukocyte-endothelial cell adhesion, transmigration, immune function and cell activation; generation of lipid mediators and/or of cytokines which regulate the function of mature cells with proinflammatory capability; interactions of mast cells with connective tissue and mucosal elements; and cell-cell interactions in relationship to the immune system. Presentations by noted experts will be followed by open discusson.

The speakers are F. Austen, R. Paoletti, R. Cotran, T. Springer, J. Pober, J. Harlan, L. Liotta, R. Murphy, S. Feinmark, S. Prescott, R. Soberman, E. Tremoli, W. Paul, R. Stevens, W. Owen, J. Nadel, P. Braquet, H. Claman, E. Unanue, T. Lee, S. Schlossman, M. Brenner, R. Geha, R. Crystal.

For further information and registration materials, please contact:
Ms. Geraldine Wolfle, Office of Program Planning and Evaluation,
National Heart, Lung, and Blood Institute, National Institutes of Health,
Building 31, Room 5A06, Bethesda, Maryland 20892,
(301) 496-9899. (NW5651)M

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Dr T C Bøg-Hansen, The Protein Laboratory,
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DK-2200 Copenhagen, Denmark, Fax +45.3183 7429

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A 12 week research project will also be undertaken in an appropriate subject.

The course begins in October 1990, lasts 9 months, and is mainly at the Institute of Psychiatry, partly at the Institute of Ophthalmology.

Applications are invited from medical and suitably qualified science graduates. Bursaries are available for students with good honours degrees.

For further details please contact **The Secretary, Department of Neuroscience, Institute of Psychiatry, De Crespigny Park, Denmark Hill, London SE5 8AF.** Tel: **071 703 5411 ext 3259.**

(4447)C

RSM, WIMPOLE STREET, LONDON

JOINT WINTER MEETING OF
ACP/BACR/RSM
29th, 30th November 1990

Symposium on "Cutaneous Melanoma"

Speakers include:
W Clark (Philadelphia), M Gore (London), R Halaban (Yale), M Herlyn (Philadelphia), J Johnson (Munich), R Kerbel (Toronto), R Mackie (Glasgow), M Mitchell (California), N Thatcher (Manchester).

Symposium on "Bladder Cancer"

Speakers include:
G D Chisholm (Edinburgh), C Fisher (London), M Knowles (Oxted), G M Mead (Southampton), R T D Oliver (London), J T Roberts (Newcastle), M Wallace (Birmingham).

Further details from: **Mrs B Cavilla, Institute of Biology, 20 Queensberry Place, London SW7 2DZ. Tel: 071-581 8333. Fax: 071-823 9409.** (4423)M

CONFERENCES/WORKSHOPS

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INTERNATIONAL CENTRE FOR GENETIC ENGINEERING AND BIOTECHNOLOGY

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RFLP IN PLANT BREEDING

Practical course, February 4-22, 1991
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Registration is limited to 18 scientists. Twelve places are reserved for scientists nominated by their National Scientific Focal Points.

Prerequisites: Participants must have a basic knowledge of plant breeding and molecular biology and be involved in research where the application of course material would be useful.

Submit resumes to Mr G Chatterjee, Administrative Officer, ICGEB, NII Campus, Shahid Jeet Singh Marg, New Delhi 110067, India. Telephone: 91-11-6862317. Telex: 3173286 Fax: 91-11-6862316.

Closing date for nominations: 31st October, 1990.

ICGEB New Delhi will provide accommodation and local transportation. Cost of travel to and from new Delhi will be borne by the participant. (W7676)V

IUB Conference on NUCLEIC ACID THERAPEUTICS

January 13-17, 1991, Clearwater Beach, Florida

ee 26 July Nature for details.

apply, please send research terests, list of relevant apers, and camera-ready or SCII abstract, to Dr Eric Wickrom, Department of Chemtry, University of South orida, Tampa, Florida 33620 SA, by September 30, 1990. x: 813-974-3203. Bitnet: kda5aa@cfrvm.

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120 YEARS AGO

The judgment of foreign nations gives the best clue to that of posterity; and it is therefore with peculiar interest that the countrymen of Mr. Darwin have watched the reception of his works in France and Germany. In the latter country his theory of the origin of species has been more or less completely accepted by those best qualified to judge, including men like Gegenbaur and Haeckel, and it has produced a complete literature of arguments and facts "für Darwin," without encountering any very serious opposition. In France, the truth of the theory is far less extensively admitted, and has been lately the subject of prolonged discussion in the Academy of Sciences. The debate on Mr. Darwin's claims has now been adjourned for three months, but so far as it was reported in our last number it furnishes much ground for reflection.

At the present time, Imperial France is, perhaps, the most conservative in science of any country in Europe. It is not, therefore, surprising that, with a few exceptions like M. Claparède, Franch naturalists refuse to accept the theory of Natural Selection, and do not see that it has already made a new epoch in human knowledge. Some, like M. Robin, object that it is not "demonstrable," and so not scientific at all; as if gravitation or the atomic theory had been, or could ever be, demonstrated like a proposition of Euclid. The Darwinian theory offers an explanation of acknowledged facts by the help of others equally indisputable, and it will only be "disproved" when it ceases to furnish an adequate explanation, or is superseded by a more simple and equally sufficient hypothesis. Meanwhile it fulfils one object of every theory, by stimulating research in all directions, and awakening new interests for the fresh investigations which it suggests.

A RUMOUR is current that the Government have refused both ships and assistance to the Royal and Royal Astronomical Societies, which have been for some time organising expeditions to observe the approaching total eclipse of the sun. We can hardly believe that the Government will thus venture to brave the opinion of all men of science and culture. It would be a direct acknowledgment that the Government cares as little for a recent position for England in science and the arts of peace as it did a little time ago for her position in the arts of war. Verily we are a nation of Philistines!
From *Nature* August 18, 1870.

100 YEARS AGO

The expression *ferment* is more frequently than not employed as the equivalent of *unorganized ferment*; consequently it is applied to the very agents which are incapable of producing fermentation proper. This has been so generally felt to be the case that several words have been coined in place of unorganized ferment, notably *zymase* and *enzyme*: the objection may be made to the former that it is indicative of vitality; the latter, however, is expressive, and serves only to indicate the vital origin of the agent, thus differentiating it from agents such as the mineral acids which act very similarly.
"The Terminology of Hydrolysis, Especially as Effected by Ferments", Henry E. Armstrong.

We have received the author's Hairless Paper-Pad Holder and Paper-Pad, issued by the Leadenhall Press. The Paper-Pad consists of a block of fifty sheets (7½ × 8¾) of smooth and cream-tinted paper mounted together on a stout piece of blotting-paper. The holder is made of light wood, and should be grasped by the left hand, the right hand being free to travel over the surface of the paper-pad which is placed on it. After each sheet is used, it is torn off and placed under the pad and so blotted, and by this means the height of the pad and holder is kept constant. For writing in railway carriages, and for reporting, this form of support will be most serviceable, and it might also be used for sketching-blocks.
From *Nature* August 21, 1890.

50 YEARS AGO

A STEP towards the elucidation of chromosome structure has resulted from the treatment of salivary gland chromosomes by solutions varying in *p*H of 1–14. M. Calvin, M. Kodani and R. Goldschmidt (*Proc. Nat. Acad. Sci.*, **26**, 340–349: 1940) find that at the highest range (*p*H = 13) serveral changes take place. Initially the chromosomes lose their striations of disks, but retain a clear outline. The chromosomes then show two beaded strings with lamp-brush connexions between the beads. Afterwards, the two strings (chromatids) synapse and then condense. Finally, the chromosomes lose the lamp-brush structure and may become invisible. It is suggested that the phenomena are in keeping with the behaviour of a partially folded and partially extended polypeptid chain. The secondary bands are broken by the high *p*H which corresponds with the iso-electrical part of nucleoprotein. The double chromonema constituting one chromatid only becomes visible when the treatment by alkali concentrates the nucleic acid upon them. Other chromonemata are fully expanded to form the loops and bristles of the lamp-brush appearance. They have been named trophochromatin and are believed to provide reserve material for growth.

Would curtailment of practical work necessarily promote the consideration of social and humanistic contacts? I fear it would unconsciously encourage a dogmatic and authoritarian attitude, by removing that constant reference to observed fact which is the distinguishing mark of science. Since this experimental attitude permeates both the theoretical outlook of science and its technical use, it is an essential part of all its social values too.

If a science teacher asks himself, at each stage in his teaching, what aims he has in view, and applies an experimental outlook to the problem of how to achieve them, he will find that some traditional practical work can be omitted. But at the same time by watching how children react if allowed to do as they please with, for example, lenses, magnets, earthworms, mechanics apparatus (provided this is large enough for the forces to be felt by the organs with which we normally estimate forces — the sensory nerves of the muscles), he will find many suggestions for pupils' practical investigations bearing on every-day life, scientific method, applications of biology, local industry, etc.
"Practical Science in Schools", E.T. Harris.
From *Nature* August 24, 1940.

10 YEARS AGO

The report of the Central Electricity Generating Board (CEGB) for the year to the end of March 1980 includes a detailed analysis of the costs of generating electricity from power stations of different types which is likely to be much quoted in the months ahead, when plans are being laid for the further development of the British nuclear power programme . . . During the past year, the cost of nuclear power generated from the first generation of British "Magnox" stations was less than that of electricity from coal or oil.

Part of the reason why the nuclear costs turn out to be so advantageous is that the initial capital costs of the Magnox stations, built between the late 1950s and the late 1960s, are much lower than present-day replacement costs would be . . . The calculations now published show that a nuclear generating plant has potential advantages over a coal-fired plant both in generating electricity more cheaply and in its effect on the economics of the generating systems as a whole.
"Nuclear generation costs compared".
From *Nature* August 21, 1980.

IN NEXT WEEK'S ISSUE

Questioning the Big Bang ■ The Vela pulsar's Christmas spectacular ■ Snails with strange eating habits ■ The *int-1* oncogene and brain development ■ Telemore involvement in ageing and other processes ■ The Hudson River's long arm

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NATURE'S 1990 CONFERENCE

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John Kappler (Denver)
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Ronald Schwartz (NIH)
hristopher Goodnow (Stanford)
Susumu Tonegawa (MIT)

2. ANTIGEN PROCESSING AND PRESENTATION

Jack Strominger (Harvard)
Don Wiley (Harvard)
Alain Townsend (Oxford)
Emil Unanue (U. Washington St Louis)

3. AUTOIMMUNITY AND CONTROL OF IMMUNE RESPONSE

Hugh McDevitt (Stanford)
Leroy Hood (Caltech)
Lawrence Steinman (Stanford)
Jacques Miller (Melbourne)
Irun Cohen (Weizmann Inst.)

4. VACCINES AND NTIBODIES

César Milstein (MRC Cambridge)
Greg Winter (MRC Cambridge)
Jay Berzofsky (NIH)
William Paul (NIH)

Commentators: Charles Janeway (Yale)
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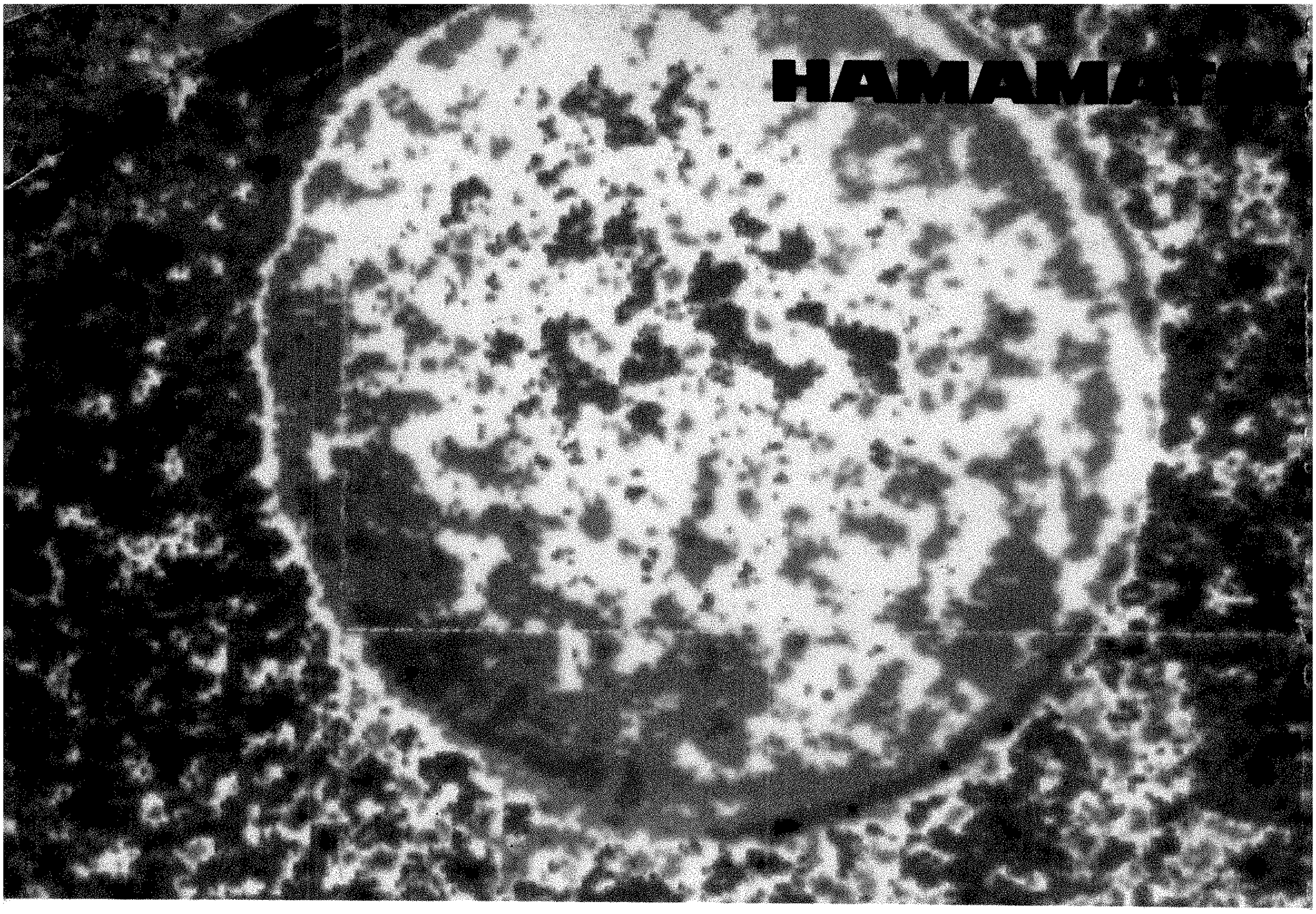

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